PHOTOSYNTHESIS

THE ASSIMILATION OF CARBON BY GREEN PLANTS

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WITH DIAGRAMS

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PREFACE

In the following pages an attempt is made to present a view of our present knowledge of Photosynthesis An earlier work on the same subject, written some eight or nine years ago by Mr I Jorgensen and the writer, was essentially a critical account of what were then recent developments of the subject The present book is not a revision of that earlier one While no attempt has been made to emphasise at all strongly the historical development of the subject, the present work is intended to be more general in its scope than that earlier one, and is not simply a critical review of recent developments, although naturally particular attention is devoted to them

Indeed, during the last decade considerable advances have been made in our knowledge of Photosynthesis, but there is not, so far as I am aware, any recent account of the question which can be described as adequate. The brief account given by Benecke in the fourth edition of Jost's well-known "Pflanzenphysiologie" is admirable as far as it goes, but does more justice to Continental than to recent British and American work, probably because of difficulties in obtaining literature It is hoped that no important work has been overlooked in the present treatment of the subject, and every care has been taken to do justice to the experimental findings and the theoretical views of the numerous writers whose work is dealt with in these pages. But it is scarcely possible that in dealing with such a vast amount of work some error should not creep in, and I should be grateful to any reader who would let me know of any such error he should detect.

The bibliography does not pretend to be complete, and is, with few exceptions, confined to works cited in the text Nevertheless, it contains upwards of 870 references, and certainly includes the vast majority of works of importance in, or bearing on, the subject of Photosynthesis that had appeared up to the autumn of 1924 Those requiring references to older literature not mentioned here

will find many such references in Pfeffer's "Physiology of Plants" and Czapek's "Biochemie der Pflanzen."

It is a pleasure to me to acknowledge the help I have received from my father, who prepared Figs. 1 and 2 for publication, and from Dr. F F. Blackman, Mr A J. Wilmott and the Council of the Royal Society, who kindly lent the blocks of Figs. 4, 5 and 6 for reproduction in these pages

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PHOTOSYNTHESIS

CHAPTER I

INTRODUCTION

THE processes taking place in the green leaf, which involve the absorption of carbon dioxide from the air and the manufacture of carbohydrates from it and the water supplied by the soil, have for a long time proved the most attractive of all the problems of plant physiology, and are among the very few problems of botany which have attracted the attention of workers in other fields This interest is undoubtedly justified, for it is upon these processes which take place in the green leaf that in the first place practically all life depends. The green plant manufactures in its leaves the material which, apart from water, forms the basis of the whole plant body, and on which the vast majority of non-green plants and practically all animals are ultimately nourished The manufacture of carbohydrates in the green leaf may be regarded as the central fact of life on this planet. Also our present civilisation is directly traceable to these same processes The substances manufactured in the leaf are substances of higher energy content than those raw materials of air and soil out of which they are built, and it is the energy stored up in this way in plants of bygone ages and which remain to us in the form of coal, that rendered possible the industrial developments of the last century.

Our knowledge of these fundamental processes taking place in the leaf commenced with the work of Joseph Priestley in the latter half of the eighteenth century. Before this time, and indeed, in certain quarters, for some considerable time afterwards, the humus theory of plant nutrition was generally accepted, according to which it was supposed that the whole of the nutrient supply of the plant was absorbed from the humus of the soil by the roots. Priestley struck the first blow towards the demolition of this theory by his famous experiments made in 1771, in which he showed that sprigs of mint under a bell-jar containing an atmosphere that had become vitiated by animal respiration, were capable of purifying

the air so that it again became capable of allowing respiration and combustion While later experiments of the same worker did not always yield uniform results (1779), he found generally that plants evolved " dephlogisticated air," that is, oxygen

The reason for the inconsistency in Priestley's results became clear from the work of Ingen-Housz (1779), who realised the importance of Priestley's discovery and extended his observations. He succeeded in showing that the purification of vitiated air by plants only takes place in the light, a fact which Priestley had not recognised, while in the dark plants contaminate the air in the same way as animals do. He also made it clear that only the green parts of plants possess the power of purifying the air

That the evolution of oxygen by plants in sunlight is accompanied by absorption of carbon dioxide was first suggested by Senebier (1783, 1788, 1800), who found that leaves exposed to light give off more " pure air " the greater the quantity of " fixed air " (carbon dioxide) contained in the water in which the leaves are submerged. Senebier, at any rate in his earlier work, was far from realising the connection between the absorption of carbon dioxide and evolution of oxygen, and Ingen-Housz (1796) is generally regarded as the first to put forward a connected theory of plant nutrition based on the fixation by plants of the carbon of carbon dioxide (cf Sachs, 1875 ; Gibson, 1914), but H Brown (1900) said of this work of Ingen-Housz : " All that is good and sound in this essay is taken from Senebier's papers without any acknowledgment, but, in appropriating ideas which he evidently understands very imperfectly, he has built up a system of plant economy which is almost unintelligible."

An advance of the first importance was made by de Saussure (1804), who sought to determine quantitatively the relation between the carbon dioxide taken in and oxygen excreted by green plants in the light. It is impossible to overestimate the importance of de Saussure's work, and the appreciation of this work by subsequent writers (*e g* Hansen, 1882 , H Brown, 1900 , Gibson, 1914) is well deserved

De Saussure's results are generally cited as showing that the same volume of oxygen is evolved as of carbon dioxide absorbed Having regard to the crudeness of his method of experimentation, this is quite a fair conclusion to draw, although de Saussure's results do not show exact equality, nor did he himself draw the conclusion that they did De Saussure also showed that the absorption of carbon dioxide by a sunflower plant was accompanied by an increase in weight, although he himself did not completely recognise the supreme importance of this discovery, and so confirmed less critical observations of Senebier indicating the fact that organic material is produced as a result of the absorption of carbon dioxide. De Saussure further showed that plants growing in sand or water, so that their only supply of carbon was

the carbon dioxide of the air, not only increased in dry weight but also in their content of carbon

While the researches of Priestley, Ingen-Housz, Senebier and de Saussure had established the fact that it is only the green parts of plants that exhibit this intake of carbon dioxide and excretion of oxygen in light, it appears to be Dutrochet who first emphasised the importance of the green pigment chlorophyll, to which the colour of green parts of plants is due Dutrochet (1837) definitely regarded the capacity for absorption of carbon dioxide on the part of the green parts of plants as directly dependent on the presence of chlorophyll

The work of Ingen-Housz, Senebier and de Saussure had made it clear that the carbon dioxide of the air is a source of the organic substance in the plant, but it was not at first realised that it is the only source of the carbon compounds of the plant, and it was due to the vigorous advocacy of this view by Liebig (1840, 1843*a*, *b*) that it came to receive general acceptation, and the humus theory fell into disrepute (cf. also Moll, 1877, 1878) When, however, it was realised that all the organic material in the plant is derived from the carbon dioxide absorbed by the leaf, it also became clear how manifold and complex must be the changes which lead up to the formation of the exceedingly complicated substances forming the body of the plant Von Mohl (1851) and Unger (1855) thought that the first products formed in the green leaf were carbohydrates, but without producing any definite proof, and it was Sachs (1862*b*, 1864*a*) who produced experimental evidence in support of the view that the starch present in green cells in the form of starch grains, previously observed by von Mohl (1837, 1845, 1855) and Nageli (1858), arose there from the carbon dioxide absorbed Sachs, indeed, spoke of starch as the first visible product of assimilation He based his opinion in the first place on the result of an investigation on the distribution of starch in plants, but he also showed that if leaves are kept in the dark for several days the starch completely disappears from them. If the leaves are then again exposed to light starch is again formed.

Subsequent investigations showed that Sachs's generalisation required some modification, for it was found that many plants do not form starch at all Thus Meyer (1885), as a result of a comparative examination of a large number of species, was able to group plants into classes according to the quantity of starch they contain. Thus members of the Gentianaceæ, most Compositæ, Umbelliferæ, and a number of Monocotyledons were found to contain no starch at all Chemical analyses of the leaves of a number of such species make it clear that in these cases sugars or a sugar is formed, and similar investigations on starch-forming plants indicate that in these also it is highly probable that the formation of starch is preceded by the production of sugar

The broad outlines of the processes we are considering are therefore clear. In the green parts of plants, that is, those organs whose cells contain chlorophyll, carbon dioxide is absorbed in presence of light, and carbohydrates are produced and oxygen evolved As suggested by the experiments of de Saussure, and as shown by more exact experiments of Boussingault (1864) and later workers, the volume of oxygen evolved is approximately equal to the volume of carbon dioxide absorbed. The equation which sums up the whole process, assuming the first product recognised to be glucose or some other hexose sugar, is therefore

$$6CO_2+6H_2O=C_6H_{12}O_6+6O_2$$

While it is possible to bring about the production of sugar from carbon dioxide and water outside the plant in more than one way, the conditions necessary for effecting this are vastly different from those which prevail in the plant It is to be noted that merely bringing chlorophyll, carbon dioxide and water together in the presence of light has not been found sufficient to effect the production of carbohydrate For this to take place it is necessary that the chlorophyll should be present in the living cell, and, in nearly all cases, in the chloroplasts. Obviously at least one other constituent of the plastid or of some other part of the cell protoplasm, or else some particular physical condition of material, is an essential factor in the process What this factor is, is at present quite unknown

We are equally ignorant of the way in which the carbon dioxide and water in the leaf are worked up into carbohydrate The work of F. F. Blackman and others has indicated clearly that there must be at least two stages in the process, one of which is dependent on light, the other of which can proceed in the dark, but although many hypotheses have been put forward to explain the mechanism of the manufacture of carbohydrates in the green parts of plants, none of these hypotheses rests on any secure basis The depth of our ignorance of this aspect of the subject is clearly indicated by the variety of terms which have been used to describe the processes: carbon assimilation, carbon dioxide assimilation, carbonic acid assimilation, photosynthesis, photosynthetic assimilation, chlorophyllous assimilation, photosyntax, photolysis of carbon dioxide, most of which assume something unproved about the nature of the processes The only terms which have remained in common use in English are carbon assimilation and photosynthesis The latter has the advantage of brevity, it has the disadvantage of presupposing a synthesis which takes place in the light It is true that light is necessary for the whole process, but it is unknown how many stages are involved in the complete process, and it is doubtful whether light is involved in the actual building up of the complex substances The term "carbon assimilation" is less objec-

tionable inasmuch as it suggests the working into the body of the plant of carbon, but objection is taken to it on the ground that "in analogy with the term 'nitrogen assimilation' it would indicate that carbon could be directly assimilated" (Ewart, 1900). This objection seems rather academic, and indeed, where no term is free from objection, it is not very material perhaps which term is selected There is certainly no reason for departing from terms in common use, and from every point of view there is little to choose between carbon assimilation and photosynthesis The present writer prefers carbon assimilation, as this term does not assume anything definite about the mechanism of the process. Photosynthesis, on the other hand, is briefer, and is therefore preferred by many Both terms are likely to endure, and both have therefore been incorporated into the title of this book

From the brief historical sketch given above of the development of our knowledge of carbon assimilation by green plants, it will be evident that several different aspects of the problem present themselves. Thus carbon assimilation first presented itself to investigators as a question of the exchange of gases between the plant and its environment The first stage of the assimilatory process is a diffusion of gas from the outer air into the cells in which the working up of the carbon dioxide and water takes place A proper understanding of how this diffusion of carbon dioxide takes place is only possible with an adequate knowledge of the system through which the diffusion must proceed A chemical investigation of the system involved is also demanded by a second aspect of the problem, namely, the participation in the process of certain substances, of which the green pigment chlorophyll appears the most obvious, but which later investigations have clearly shown is not the only one The early observation that light is an essential for photosynthesis suggests a third aspect of the problem, the influence of external conditions, of which light was the first to be recognised, but which may include others, as, for example, temperature. The work of Sachs suggests a fourth aspect of the problem, namely, the nature of the products of assimilation Since the production of these substances involves the absorption of energy which is provided in the form of radiant energy from the sun, the energy relations in photosynthesis constitute a fifth aspect of the problem of carbon assimilation A sixth aspect is what is generally termed the mechanism of carbon assimilation, that is, the chemical changes involved in passing from water and carbon dioxide to carbohydrates. This is the aspect of the subject of which our ignorance is most complete. Lastly, there is the question of how the assimilatory process is related and linked up with other processes in the plant, translocation of food material, transport of water, nitrogen metabolism, respiration, and so on This is an aspect of the subject which is obviously of great importance in regard to the life of the plant as a whole, but our knowledge of the various problems

concerned is very varied, quite considerable in some cases, in others of the slightest

In the following chapters these various aspects of the problems of the assimilation of carbon in green plants are discussed, and an attempt is made to exhibit the present position of our knowledge with regard to them.

CHAPTER II

THE SYSTEM INVOLVED IN THE ASSIMILATING CELL

The Assimilating Organs

The green parts of plants, those in which the photosynthetic processes take place, may be spoken of for the sake of brevity as assimilating organs While these may differ widely in structure in different groups of the plant kingdom, and even sometimes among closely related species, and, indeed, in different parts of the same individual, the *chief* assimilating organs of the plant are characteristically thin expanded structures, such as the leaves of vascular plants and mosses, the thalli of many liverworts and the expanded laminæ of many of the larger algæ Departure from this characteristic form is met with chiefly in connection with habitat conditions, as in the case of many xerophytes, where a reduction in surface is correlated with the necessity for keeping down evaporation of water, and in organs such as stems and leaf-stalks in which assimilation is only a secondary function of the organ

This characteristic shape of the assimilatory organs is clearly related to their function Carbon dioxide is absorbed from the atmosphere, and the radiant energy of sunlight is also absorbed. By means of a thin expanded structure a comparatively small quantity of plant material is required for providing a considerable absorbing surface The thicker the organ, the smaller the quantity of energy reaching the middle of it, and the further the carbon dioxide will have to diffuse While a leaf one cell thick would provide the maximum surface for a given quantity of material, such thin laminæ, although found in the mosses and a very few vascular plants, are very unusual among these latter in which various conditions, such as the provision of a conducting system and external mechanical influences, such as wind and rain, impose a limit on the possible thinness of the leaf

In fresh-water and marine algæ the carbon dioxide must diffuse in aqueous solution through the external walls of the outer cells of the assimilating organs, and the same must be true of the leaves of mosses where there is no stomatal system In completely submerged flowering plants the leaves of which are surrounded by an aqueous medium, and many of which do not possess stomata, as,

for example, *Elodea* and *Vallisneria*, the same must be the case In all subaerial pteridophyta and higher plants, however, the possession by the shoot of a system of stomata opening into intercellular spaces renders possible the diffusion of atmospheric gases throughout the intercellular spaces of the leaf, so that it is possible that internal cells of the leaf may absorb carbon dioxide direct from the air in the lacunar system through their own surfaces, and not by way of the surface cells. In any case carbon dioxide must diffuse into the assimilating cells in solution in the water in the cell wall.

Considerable variation in internal structure of the assimilating organs exists even in plants of the same group. Among the higher plants the cells of the outer layer of the leaf, the upper epidermis and the lower epidermis, are as a rule devoid of chlorophyll with the exception of the guard cells of the stomata The assimilating tissue of the mesophyll is most generally differentiated into the compact palisade tissue on the morphologically upper surface, and the looser spongy tissue on the lower side Where the mesophyll is so differentiated, considerable differences exist among different species in regard to the relative quantities of palisade and spongy tissue, the size of the cells, and so on. It is well known that in some cases such differences can be induced in plants of the same species by exposing them to different external conditions, whereas other species are more resistant to such changes of development (Stahl, 1880*b*, Clements, 1905). It will be of interest for us to inquire later how far such variations in structure affect assimilation.[1]

The Assimilating Cell

The assimilating cell itself, whether belonging to palisade, spongy tissue or to undifferentiated mesophyll of a higher plant, or whether an assimilating cell of a moss, liverwort or alga, has certain characteristics Except in a few free-swimming forms, it is bounded externally by a cell wall of complex composition containing celluloses, pectins and possibly fatty substances held together in some way not properly understood, but perhaps as a hydrogel, but in any case containing a considerable quantity of water. Immediately within the cell wall is a layer of protoplasm which may or may not possess strands stretching across the central vacuole within the limiting cytoplasmic layer In addition to the nucleus there are present the chloroplasts or chromatophores, the bodies in which the green pigment is localised These are completely surrounded by cytoplasm, and do not come into immediate contact with cell wall or vacuole. There are also present, at least in some assimilating cells, small granules termed chondriosomes or mitochondria

As it is in these cells, and in these cells only, that the assimilatory processes take place, a knowledge of their structure and composition

[1] For recent descriptions of assimilatory tissue see Budde (1923) and Zincke (1924).

is clearly likely to be of the utmost importance if the assimilatory processes are to be understood This holds particularly in regard to the bodies which contain the green pigment While very considerable advances have been made during the last twenty years in regard to certain parts of the system involved in the assimilating cell, it is equally true that very little is known about other parts of the system which may be of the greatest importance in the processes of assimilation

The Cytoplasm

The consensus of recent opinion, derived from observations with the microscope and ultramicroscope and by means of microdissection, is that the cytoplasm of active cells, including assimilating cells, is a hydrosol (Bayliss, 1915, Seifriz, 1921). Observations with regard to the chemical composition of this cytoplasmic sol in assimilating cells, apart from microchemical tests, are lacking, and it is clearly going to be no easy matter to make even an approximate quantitative chemical analysis of the cytoplasm of green leaves. While it is generally realised now that cytoplasm is not one substance or constant group of substances throughout the plant kingdom, but a very complex mixture of substances (a polyphase hydrosol) varying from species to species and perhaps from cell to cell in the same individual, and from time to time in the same cell, it appears to be generally supposed that different cytoplasms are mixtures of the same sort of things While such vagueness may be very regrettable, we can, under the circumstances, do no more than accept such analyses of protoplasm as are on record. The best known of these is that of Reinke (1881*a*, 1883*a*) and Reinke and Rodewald (1881), of the plasmodium of the myxomycete *Æthalium septicum* (=*Fuligo varians*), which consists largely of cytoplasm and nuclei, as suggestive of the composition of the cytoplasm of higher plants It must nevertheless be borne in mind that the plasmodium of a myxomycete is a very different thing from the assimilating cell of a higher plant, also the analyses only relate to the composition of killed plasmodia The results given in Table I must therefore be regarded in relation to these facts

TABLE I

Analysis of the Plasmodium of Æthalium septicum (=Fuligo varians)

(Data from Reinke and Rodewald)

Substance	Percentage of dry weight
Proteins	40·0
Albumins and enzymes	15·0
Other nitrogenous compounds	2·0
Fats	12·0
Carbohydrates	12 0
Cholesterol	2 0
Resins	1 2
Calcium salts (except calcium carbonate)	0 5
Other salts	6 5
Undetermined matter	6 5

Recently the question of the composition of protoplasm has been reopened by Lepeschkin (1923), who rightly points out that at the time when the analyses of Reinke and Rodewald were made little was known of the composition of proteins, and especially of nucleo-proteins Lepeschkin has therefore analysed the plasmodium of a myxomycete which appears to be probably *Fuligo varians*, with the result recorded in Table 2 The water content of the plasmodium amounted to 82·6 per cent

TABLE 2

ANALYSIS OF THE PLASMODIUM OF A MYXOMYCETE RESEMBLING FULIGO VARIANS

(Data from Lepeschkin)

Substance	Percentage of dry weight
A. Water-soluble organic substances chiefly contained in the vacuoles	
Monosaccharides	14 2
Proteins	2 2
Amino-acids, purine bases, asparagin, etc	24 3
B. Insoluble organic substances which principally form the ground mass of the protoplasm	
Nucleo-proteins	32 3
Free nucleic acids	2 5
Globulin	0 5
Lipo-proteins (plasmatin)	4 8
Neutral fats	6 8
Phytosterol	3 2
Phosphatides	1·3
Other organic matter (polysaccharides, pigments, resins)	3 5
C. Mineral matter, of which about half is extractable with water	4 4

For a very detailed account of the structure of the cell and cytoplasm, reference should be made to the recent work of Lundegårdh (1922*a*) on this subject

THE NUCLEUS

Very little that is definite is known concerning the composition of the nucleus, nor, indeed, of its function in the vegetative cell in general, although its presence is essential Ultramicroscopic observations by Price (1914) suggest that it is in the gel state It is said to consist largely of proteins containing phosphorus, while potassium is stated to enter constantly into its composition The part it plays, if any, in the photosynthetic process, is obscure in the extreme

THE CHONDRIOSOMES

The presence of small filamentous rod-like and coiled granules in plant cells resembling similar bodies previously observed in

animal cells and termed chondriosomes, was first notified by Meves (1904) as occurring in the tapetal cells of the young anthers of *Nymphæa* (=*Castalia*) *alba* Since then they have been recorded as occurring in the vegetative cells of stem, leaf and root of various higher plants, as well as in various mosses (Sapehin, 1913), *Vaucheria* (Rudolph, 1912*a*, *b*) and fungi (Guilliermond, 1911*a*, 1913, *a*, *b*, *c*). The chief interest of chondriosomes from our present point of view lies in the fact that it has been urged that chondriosomes give rise to plastids, including chloroplasts, of higher plants

The Chloroplasts

The chloroplasts are the bodies, included within the cytoplasm of the assimilating cells, which contain chlorophyll and are therefore coloured green. They differ greatly in size and shape in different species In the higher plants they are very generally small ellipsoidal or disk-shaped structures, of which there are a considerable number in a single cell, and the same is also the case in the Bryophyta The chloroplasts of *Selaginella* are usually large, and may possess a diameter as great as 0 02 mm , most of the chloroplasts of vascular plants are considerably smaller In 215 species examined by Mobius (1920), the diameter of the chloroplasts varied between 3μ and 10μ, and in only 9 species did the diameter exceed 7μ. The chromatophores of the Phæophyceæ examined by Senn (1919) were also found to vary between 3μ and 7μ , but in the green algæ, on the other hand, there are many species in which the cells contain a few large chloroplasts, or only one. The filamentous algal genera *Spirogyra*, *Mougeotia*, *Zygnema* and *Ulothrix* are well-known examples of such

It has been emphasised that chloroplasts can be converted into colourless leucoplasts or orange or red coloured chromoplasts by the loss of all pigment in the former case, and by development of a red pigment in the latter, with destruction or masking of the green pigment The only difference between the various plastids lies in the presence or absence of a particular pigment, and the change from one to the other can often be brought about at will Thus in autumn leaves the green chloroplasts become changed into the yellow or red chromoplasts, while the leucoplasts in organs not normally exposed to light, as, for example, potato tubers, will develop chlorophyll when so exposed. By treatment of the chloroplast with alcohol or some other solvent the green pigment can be removed, leaving behind the colourless body of the plastid, the so-called " stroma "

A more controversial question is that of the origin of the plastids. It was urged by Meyer (1883*a*, *b*) and Schimper (1883) that plastids always arise by division of pre-existing plastids, and never arise *de novo* from the cytoplasm This view received general support from botanists, and will be found stated as a fact in many text-

books (see, for example, Pfeffer, 1900). In 1910 and 1911, however, Lewitsky, as a result of cytological studies of various tissues of *Pisum* and *Asparagus*, came to the conclusion that the chondriosomes in the cells of the root apex develop into leucoplasts, and those in the shoot apex into chloroplasts. Similar conclusions were drawn by Pensa (1910), Forenbacher (1911) and Guilliermond (1911*b*, *c*, 1912*a*, *b*, *c*, *d*), from observations on a number of other species. If these workers have interpreted their experimental observations correctly, it can apparently refer only to higher plants, for various investigators on mosses, liverworts and algæ have traced the chloroplasts through the whole life cycle, and have shown that they always arise from pre-existing plastids, whether chondriosomes are present or not

From the point of view of an understanding of the physiology of the plant in general, the origin of the plastids may well be an important question, but in regard to carbon assimilation there is no need to enter further into the controversy of the origin of the chloroplasts What is more important is to obtain some knowledge of the structure and composition of these bodies, as the localisation in them of the pigment without which photosynthesis cannot take place indicates the probability that they play an important part in assimilation

While the chloroplasts are generally regarded as protoplasmic structures, they are nevertheless differentiated very definitely from the cytoplasm From a chemical point of view, as we have already noted, they can be separated into two parts, the pigment and the colourless body or stroma. With the pigments of the chloroplast we shall deal in the following chapter While our knowledge of the chemistry of the assimilatory pigments is now considerable, our knowledge of the chemistry of the stroma is very vague Proteins are supposed to enter largely into the composition of the colourless stroma of the plastid (cf Zacharias, 1883, Zimmermann, 1887, 1893–1894), and although it has been supposed that the protein is a temporary food reserve (cf Meyer, 1915 1917*b*, 1918*a*, *c*), there is strong evidence that much of the protein of the plastid is not to be reckoned such (cf Ullrich, 1924) Fats and other lipoid substances appear to be constantly present, sometimes in considerable amount (Zimmerman, *l c.*, Schmitz, 1882, Engelmann, 1883, Schimper, 1885*a*, Meyer, 1885, Reinke, 1885*c*, Hansen, 1889, Buscaloni, 1912) It is much to be desired that a thorough chemical investigation of the colourless part of the chloroplast should be made, as it must be regarded as extremely probable that some of the colourless substances of the chloroplast play an important part in photosynthesis That fixing agents and other poisons appear to affect the chloroplasts very much less than they affect the protoplasm indicates that the latter differs very considerably from the plastids in composition

With regard to the physical structure of the chloroplast very

different views have been held. According to the "grana" theory of Meyer (1883*a*, *b*), the stroma consists of a kind of sponge-like porous skeleton in the interstices of which are fatty pigment-containing drops or "grana." This opinion rested particularly on the visible structure of the chloroplasts of certain orchids, and there appears to be no evidence that such an appearance is general. According to Liebaldt (1913), a granular appearance of the uninjured chloroplast is only observable when it contains numerous small inclusions of starch or fat which arise there as temporary reserves during the photosynthesis process Further, according to Kuster (1911) and Liebaldt (*l.c*), the chloroplast may vary considerably in consistency, and instead of being rather a solid object, as Meyer's theory would suggest, is rather of liquid nature, especially in the Orchidaceæ and Florideæ. Lepeschkin (1910) came to a similar conclusion with regard to the chromatophore of *Spirogyra*.

In this connection the ultramicroscopic observations on the chloroplast made by Price (1914) are of particular interest. He states that the chloroplast usually appears as a slightly opaque and heterogeneous body with a motionless gel structure, there being no moving particles in it even when the surrounding cytoplasm is filled with these. In *Elodea* the chloroplasts move in the streaming protoplasm, but not so rapidly as the cytoplasm.

The colloidal nature of the stroma is probably now generally accepted (cf Lepeschkin, 1911, Ponomarew, 1914). Thus Liebaldt (1913) thought the fatty part of the plastid which contains the pigment is distributed in colloidal form through the hydrocolloid, probably protein in character, which constitutes the stroma

The pigment to which the colour of the chloroplast is due constitutes only a small proportion of the mass of the whole body of the chloroplast, and considerable discussion has arisen regarding the way in which the pigment is held and distributed in the plastid The "grana" theory of Meyer has already been noticed Pringsheim (1881*c*) and Wager (1906), from microscopical examination of killed and living chloroplasts respectively, concluded that the pigment is more or less uniformly distributed throughout the delicate meshes of the colourless stroma, Timiriazeff (1903) by microscopical examination of the chloroplasts in red light, and Priestley and Miss Irving (1907), partly from experiments in which chloroplasts were allowed to split in water or dilute sugar solution, and partly from examination of sections 1μ thick of fixed and unfixed chloroplasts cut by means of a microtome, concluded that the chlorophyll is confined to the periphery of the plastid, where it is held in the meshes of a network According to Czapek (1922), however, this appearance is probably artificially produced as a result of swelling

It is now regarded as possible that the chlorophyll is adsorbed at the surface of colloidal particles in the colourless stroma, and that it is itself present in the colloidal condition Evidence for

For a further discussion of the movements of chloroplasts in response to changes of light intensity reference may be made to Pfeffer (1906) and the literature cited therein, and to the more recent papers by Senn mentioned above.

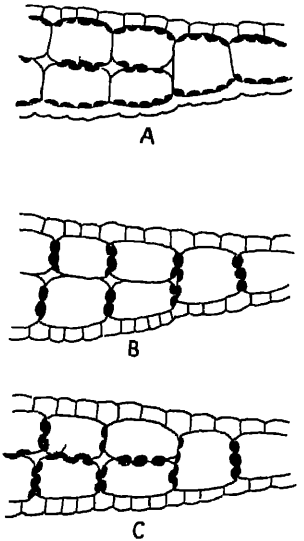

FIG. 2.—Transverse sections through the leaf of *Lemna trisulca* showing the position assumed by the chloroplasts in (A) light of moderate intensity, (B) intense light, and (C) darkness (After Stahl)

THE VACUOLE

Within the cytoplasm is contained the vacuole, from which it may perhaps be separated by a boundary layer, the inner plasmatic membrane, tonoplast or vacuole wall, having properties differing from those of either cytoplasm or vacuole While colloidal, and even solid, particles may be present in the vacuole, this consists chiefly of an aqueous solution of various substances, as the facts of osmotic pressure and turgor of the cells sufficiently indicate In the assimilating cells the vacuoles may thus form a place of temporary storage of the soluble sugar formed in photosynthesis

In red leaves, such as those of the copper beech, and in the young leaves of many plants, anthocyanin pigments are present dissolved in the cell sap contained in the vacuole Such pigments have nothing to do with the photosynthetic process, and will not therefore be considered in the following chapter on the pigments of the assimilatory organs.

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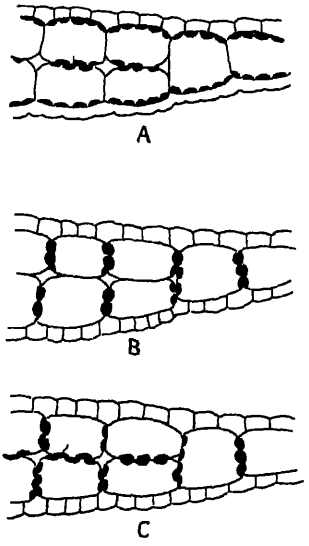

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CHAPTER III

THE ASSIMILATORY PIGMENTS

General Remarks

The substances to which the colour of the green leaf is due have for long formed a favourite subject of investigation The name "chlorophyll" appears to have been first applied to the colouring matter of the green leaf by Pelletier and Caventou in 1818, but long before this, in 1682 in fact, Grew recorded the extraction of green and yellow pigments from leaves by means of oil, indicating even at that comparatively early date that there may be more than one coloured substance in the leaf The first definite assertion to this effect appears to be that of Frémy (1860), who treated the residue from the alcoholic extract of leaves with strong hydrochloric acid and ether, and observed that the ether became coloured yellow and the aqueous acid yellow-blue. Frémy called the blue substance phyllocyanine and the yellow ether-soluble one phylloxanthine Treatment in the same manner of the precipitate produced by adding aluminium hydroxide to the alcoholic extract gave the same result Frémy concluded that there is a blue-green and also a yellow pigment present in chlorophyll

Stokes (1864*a*), while holding the view that the pigments separated by Frémy were mainly decomposition products of chlorophyll resulting from treatment with acid, decided that there are actually four different pigments in the leaf, two green and two yellow The method he adopted to separate the pigments, that of partition between non-miscible solvents, was really involved in Frémy's method of separation Although details of Stokes's work appear never to have been published, he said (1864*b*), "Bisulphide of carbon in conjunction with alcohol enabled the lecturer to disentangle the coloured substances which are mixed together in the green colouring matter of leaves" This method has proved of great importance in subsequent attempts to separate the leaf pigments Of the pigments so separated Stokes said: "I find the chlorophyll of land plants to be a mixture of four substances, two green and two yellow, all possessing highly distinctive optical properties The green substances yield solutions exhibiting a strong red fluorescence, the yellow substances do not The four substances are soluble in

the same solvents, and three of them are extremely easily decomposed by acids or even acid salts, such as bis-oxalate of potash, but by proper treatment each may be obtained in a state of very approximate isolation so far at least as coloured substances are concerned."

Isolation of yellow pigment from alcoholic extracts of leaves was later effected by Frémy (1865) by the hydroxide of magnesium aluminium, calcium or barium, or by precipitating all the pigments by addition of barium hydroxide and then extracting yellow pigment from the precipitate by means of alcohol This result was confirmed a few years later by Timiriazeff (1871), who gave the name xanthophyll to the extracted yellow pigment, a name already given by Berzelius (1837*a*, *b*) to the yellow pigment extracted by him from autumn leaves of the pear The same term was employed by Sorby (1871) for yellow pigment of the leaf.

In the following year Kraus (1872) also separated a green and yellow pigment from the alcoholic extract of leaves by shaking such an extract with benzene, when the benzene became coloured green, leaving the alcohol yellow. Kraus also termed the yellow pigment xanthophyll, but the green pigment he called cyanophyll. Kraus's work was confirmed by Konrad (1872), who noted that the separation could only be brought about if the alcohol contained water, 80 per cent alcohol giving the best result Subsequent workers (Cempert, 1872, Treub, 1874, Wiesner, 1874*a*, *b*) found that benzene could be replaced by various solvents in the separation of the pigments

In spite of these researches we yet find that Hansen in 1882 was very sceptical of the presence of both green and yellow pigments in the chloroplasts Two years later, however, he separated the green and yellow components himself, and called them chlorophyll green and chlorophyll yellow.

Sorby (1873), using the method suggested by Stokes, thought he had separated two chlorophylls and five xanthophylls, but it seems clear now that Sorby did not succeed in a complete separation of the different leaf pigments, and that some of his supposed pigments were mixtures.

Dippel (1878) by spectroscopic observations found that Kraus's cyanophyll contained a yellow pigment different from the xanthophyll, but the confirmation of Stokes's discovery of two yellow pigments in the leaf was first due to Borodin (1883), who succeeded in isolating crystals belonging to two groups of yellow pigments, one characterised by great solubility in alcohol and slight solubility in benzene, the other by a high solubility in benzene and slight solubility in alcohol Largely by the use of the spectroscope, Tschirch (1884, 1885, 1887, 1896, 1904) also distinguished two yellow pigments, while by the same method Schunck (1899, 1901, 1903) concluded that there are present in the chloroplasts a number of yellow pigments which can be identified by their spectra Monte-

verde (1893) also found two groups of yellow pigments in the leaf, differing from one another in their relative solubilities in alcohol and petrol ether It is not very clear how far the pigments isolated by these different workers are identical or pure (cf Kohl, 1902, Palmer, 1922)

While there was thus considerable difference of opinion regarding the number of yellow pigments to be met with in the chloroplast, it appears to have been generally taken for granted that there was only one green pigment Nevertheless, Gautier (1886, 1895, 1909) expressed the view that the green pigment is different in ray-grass and spinach, while Etard (1895, 1906) supposed that a number of different chlorophylls are present in one species and an indefinite number of different chlorophylls in different species

In the early years of the twentieth century the literature of the pigments of the chloroplast thus showed that knowledge of them was in a very confused state Tswett contributed largely towards the removal of this confusion. Tswett's chromatographic method (1906*b*, *c*) of demonstrating the different pigments present in the leaf is based on the different adsorption affinities of the various pigments for calcium carbonate or other adsorbent It is very simple and can easily be repeated in the laboratory It consists in filtering a benzene, petrol ether or carbon disulphide extract of the pigments free from water through a column of dry calcium carbonate, inulin or sugar, contained in a glass tube Owing to the different adsorption affinities of the different pigments for the adsorbent, the column of the latter becomes differentiated into different-coloured layers, the different pigments being adsorbed successively This stratified column is called by Tswett a chromatogram The various layers can be separated from one another by a knife, and the pigments contained within them extracted with various solvents In this way Tswett separated two chlorophyll components which he called α-chlorophyllin and β-chlorophyllin, and five yellow pigments, called by him collectively carotinoids, and named, in ascending order of their adsorption: carotin (not adsorbed at all), xanthophyll α, xanthophyll α', xanthophyll α'' and xanthophyll β These pigments all have characteristic spectra (Tswett, 1907*a*, *b*, 1908*a*, *b*, *c*, *d*) The existence of these five carotinoids in the perianth leaves of a polyanthus narcissus and a deeply pigmented daffodil (" King Alfred ") has been confirmed by Miss Coward (1924*a*) The xanthophyll pigments are very similar to one another, and presumably belong to one chemical group If, then, for Stokes's two yellow pigments we understand groups of nearly related pigments, Tswett's results afforded a complete confirmation of Stokes

The final stage in the determination of the pigments of the leaf is the work of Willstatter and numerous collaborators, who have succeeded in isolating the various pigments in a pure state, and in determining their chemical composition and properties These

researches, conducted on a large scale and carried out over a period of more than ten years, have succeeded in solving the problem of the composition of the leaf pigments, a problem which had baffled investigators for more than fifty years. Willstatter's work is undoubtedly to be regarded as one of the greatest achievements of biochemistry. It has rendered our knowledge of the chemistry of the leaf pigments as sure as that of any other plant substance, and will no doubt prove of great importance in plant physiology.

Willstatter made a large number of analyses of the pigments in the leaves of different species belonging to a variety of families, from plants growing under a variety of conditions and in leaves collected at different times of the day. It was found that the pigments were the same in all the plants examined.

According to Willstatter the pigments of the leaf are four in number, namely—

1. Chlorophyll *a*, $C_{55}H_{72}O_5N_4Mg$, a microcrystalline blue-black solid, green-blue in solution This appears to be identical with Tswett's α-chlorophyllin.
2. Chlorophyll *b*, $C_{55}H_{70}O_6N_4Mg$, a microcrystalline green-black solid, pure green in solution This appears to correspond with Tswett's β-chlorophyllin
3. Carotin, $C_{40}H_{56}$, an orange-red crystalline substance identical with the carotin of Tswett and that isolated from carrots.
4. Xanthophyll, $C_{40}H_{56}O_2$, obtained as yellow crystals Tswett (1910) thought that the xanthophyll isolated by Willstatter and Mieg (1907) from nettle leaves was a mixture of two or three xanthophylls, but principally xanthophyll α. Miss Coward's results support this conclusion Willstatter and Stoll (1913) think this may be the case, but Palmer (1922), judging from the properties of the substances, including their spectra, thinks that Tswett's xanthophyll β was present in considerable quantity in Willstatter and Mieg's preparation Willstatter and Stoll also point out the possibility that some of Tswett's supposed xanthophylls may be oxidation products It seems to be generally agreed that if there are several xanthophylls they form a group of isomeric or isomorphic substances possessing the same empirical formula, that found by Willstatter and Mieg.

In the following pages the term "chlorophyll" will be used to designate the mixture of chlorophyll *a* and chlorophyll *b* occurring in the chloroplast, and the term "carotinoids" to indicate the yellow pigments By xanthophyll is to be understood the pigments possessing the general formula $C_{40}H_{56}O_2$

The average quantities of these various pigments present in fresh and dried leaves are indicated in the following table:—

TABLE 3

QUANTITIES OF GREEN AND YELLOW PIGMENTS PRESENT IN LEAVES
(Data from Willstätter and Stoll.)

Pigment.	Parts per thousand of fresh leaves	Parts per thousand of dry weight.
Chlorophyll *a*	2 0	6 3
Chlorophyll *b*	0·75	2 4
Carotin	0·17	0 5
Xanthophyll	0 33	0 9

Willstätter extended his observations on the plastids to those of the green and brown algæ As an example of the former he chose *Ulva lactuca*. The same four pigments were found present here, but the total quantity both of green and yellow pigments per unit weight of thallus is considerably less than that in the same weight of green leaves of higher plants. *Ulva* also contains comparatively more chlorophyll *b* in relation to the total quantity of green pigments than do the higher plants. The results of Willstatter's analysis are shown in Table 4.

TABLE 4

QUANTITIES OF GREEN AND YELLOW PIGMENTS PRESENT IN *Ulva lactuca*
(Data from Willstätter and Stoll)

Pigment	Parts per thousand of fresh weight	Parts per thousand of dry weight
Chlorophyll *a*	0 165	0 936
Chlorophyll *b*	0 117	0 666
Carotin	0 024	0 138
Xanthophyll	0 064	0 365

In the brown algæ the state of affairs is very different. It has long been known that the brown colour of these forms is due to the presence of a brown pigment in the plastid Various opinions were expressed with regard to the nature of this brown pigment. Thus Cohn (1865, 1867) thought the brown pigment, " phæophyll," was nearly related to chlorophyll, a view which was apparently shared by Molisch (1905) Millardet (1869) found that a brown pigment, soluble in water, can be extracted from members of the Phœophyceæ, and gave to it the name of phycophæin It was suggested by Reinke (1886) that this pigment might only be present in dead plants, and Molisch showed that this is the case, a water-soluble brown pigment being only extractable from dried plants or those which have been dipped in hot water The green colour remaining in the thallus after the latter treatment is due, according to Molisch, to the change of phæophyll into a green chlorophyll derivative, so that both the green and brown pigments obtained from plants so treated are post-mortal products

It has been more usual to suppose that the brown algæ contain chlorophyll, but that the presence of this is marked by the brown

pigment This view appears to date from Rosanoff (1867), and found distinguished supporters such as Tswett (1905, 1906*a*, 1910) and Czapek (1911). The brown pigment was supposed by Sorby (1873), as a result of spectroscopic observations, to be a pigment differing from the yellow pigments of higher plants, and he gave to it the name fucoxanthin Although the presence of this special pigment, and also that of carotin, was denied by Gaidukov (1903*c*), Tswett (1906*a*), by his chromatographic method, showed the presence of three carotinoids, fucoxanthin, xanthophyll and carotin Tswett also found two chlorophylls present, one, α-chlorophyllin, which also occurs in higher plants, the other, γ-chlorophyllin, which does not

The conclusion of Sorby and Tswett regarding the presence of fucoxanthin was confirmed by Willstatter and Page (1914), who isolated this substance and assigned to it an empirical formula of $C_{40}H_{54}O_6$, but the observation with regard to the presence of a special chlorophyll in the brown algæ was not confirmed by them Indeed, in *Fucus* the chlorophyll present is nearly all chlorophyll *a*, not more than 3 per cent of the whole green pigment being chlorophyll *b* The supposed third chlorophyll could, however, be obtained if stale or dried thallus were employed, and this substance thus appears to be a post-mortal derivative of chlorophyll *a*.

Spectrum analysis confirms the chemical analysis of Willstatter and Page, and shows that the spectra of the brown derivatives of chlorophyll are quite different from that of chlorophyll, whereas the spectrum of the thalli of brown algæ is little different from that of the green leaf

Different brown algæ exhibit considerable differences in the content of the various pigments Very little chlorophyll *b* is present, while considerably more yellow pigment is present than in land plants The results of analyses of Willstatter and Page are shown in Table 5 The parts per thousand of the fresh thallus are here recorded, the quantities in the dry matter can be calculated for *Fucus* and *Laminaria* by knowing that the dry weights of the thalli of these plants form respectively 28 5 and 15 4 per cent of the fresh weight

TABLE 5

QUANTITIES OF GREEN AND YELLOW PIGMENTS PRESENT IN BROWN ALGÆ

Pigment	Parts per thousand of fresh weight in Fucus	Dictyota	Laminaria
Chlorophyll	0 528	0 640	0·185
Fucoxanthin	0 169	0 250	0 081
Carotin	0 089	0 057	0 006
Xanthophyll	0 087	0 063	0 038

It will be observed that extremely little carotin is present in *Laminaria*, which accounts for the fact that Czapek (1911), by the

use of Tswett's chromatographic method of analysis, could find no carotin present in the carefully dried thallus

Information with regard to the plastid pigments of the red algæ is much vaguer than in the case of the brown algæ, and no complete quantitative analysis has been made in any case. From investigations by Sorby (1873), Reinke (1876), Nebelung (1878), Hansen (1893), Tammes (1900), Kylin (1911) and Van Wisselingh (1915), there can be no doubt that in addition to chlorophyll (see, *e g*, Noll, 1888*b*), yellow pigments are always present in the red algæ. The presence of both carotin and xanthophyll has been demonstrated, and it is possible that fucoxanthin is also present The most conspicuous pigment in these algæ is, however, the red pigment to which the name phycoerythrin was given by Kutzing in 1843 What the actual composition of this pigment may be is at present in doubt It is certainly not a carotinoid, and is generally stated to be a protein or protein-like substance (Hansen, 1893; Molisch, 1894) Hanson (1909), working with the pigment extracted from *Ceramium rubrum*, concluded that phycoerythrin is probably a colloidal nitrogenous substance related to protein, but is probably not a true protein as its nitrogen content is too low, and it does not give the biuret reaction. Kylin (1910), who also examined it particularly in the case of the same species, regards phycoerythrin as a protein containing both an albumin and a pigment group in the molecule It seems possible, however, that the composition of the red pigment may vary in composition among the Rhodophyceæ (Kylin, 1912*a*)

In some of the red algæ there is also present a blue pigment, phycocyan or phycocyanin, as, for example, in *Bangia fusco-purpurea* (Noll, 1888*b*) and *Ceramium rubrum* (Kylin, 1910)

In the blue-green algæ the blue phycocyanin is present in addition to the usual green and yellow pigments Phycocyanin was isolated by Molisch (1895), who concluded that it, like phycoerythrin, is a protein substance Some blue-green algæ develop phycoerythrin, but it appears that it may not be identical with that of the red algæ (Boresch, 1921*d*), which, as just mentioned, perhaps do not all contain the same modification of the pigment

Chlorophyll

As already mentioned, the green pigments of the chloroplast, chlorophyll *a* and chlorophyll *b*, are two very nearly related substances containing no phosphorus nor iron as was at one time thought, but containing 2 7 per cent of the weight magnesium, the only metal present They form microcrystals in the solid state, chlorophyll *a* presenting a blue-black colour and chlorophyll *b* a green to green-black colour

Chlorophyll *a* dissolves readily in ethyl alcohol, acetone, ether, chloroform, carbon disulphide, pyridine and benzene, it is

moderately soluble in methyl alcohol and soluble with difficulty in an 80 per cent aqueous solution of ethyl alcohol, a 90 per cent. solution of methyl alcohol, warm or cold, and petrol ether, warm or cold In 80 per cent methyl alcohol it is practically insoluble. The solubility properties of chlorophyll *b* are much the same as those of chlorophyll *a*, but the former is generally somewhat less soluble than the latter It is practically insoluble in 90 per cent methyl alcohol and completely insoluble in petrol ether

In solution in ethyl alcohol chlorophyll *a* is blue-green with a deep red fluorescence The alcoholic solution of chlorophyll *b* is greener with a very slight yellow tinge as compared with chlorophyll *a*, and the red fluorescence is tinged with brown In colloidal solution in water both show a little opalescence, but no fluorescence, and appear pure green.

The chlorophylls are neutral substances which are acted upon by both acids and alkalies On treatment with oxalic acid or dilute hydrochloric acid, chlorophyll yields a wax-like olive-green substance called phæophytin, chlorophyll *a* giving phæophytin *a*, and chlorophyll *b* giving phæophytin *b* The phæophytins contain no magnesium, this being replaced by hydrogen, the replacement being the only change which takes place. By treatment of phæophytin with copper acetate or zinc acetate, a metal can be reintroduced into the molecule, and copper chlorophyll [1] or zinc chlorophyll is formed, the copper or zinc occupying the position occupied originally by magnesium in the chlorophyll molecule It is not so easy to re-introduce the magnesium, but this has been done by Willstatter by treatment of phæophytin with magnesium methyl iodode. Alkali metal chlorophylls are still more difficult to produce and are less stable, the potassium compound being the least stable.

On treatment of phæophytin with alkali, there is produced, as well as nitrogen-containing acids, a nitrogen-free alcohol called phytol and possessing the formula $C_{20}H_{39}OH$. It will be observed that the phytol constitutes one-third of the weight of chlorophyll The same phytol is yielded by both chlorophyll *a* and chlorophyll *b*

On treatment with alkalies in the cold, chlorophyll is saponified like an ester, yielding salts of acids called chlorophyllins These chlorophyllin salts are green in colour but are soluble in water. The chlorophyllins contain magnesium, which, like that in the chlorophyll, is bound to the nitrogen in a complex way, the magnesium cannot, for instance, be electrolytically dissociated as in a magnesium salt

On treatment now with hot concentrated alkalies, a series of

[1] That is, copper chlorophyll *a* or copper chlorophyll *b* as the case may be In all that follows where chlorophyll or a chlorophyll derivative is mentioned, the remarks apply equally to chlorophyll *a* or chlorophyll *b*, and the derivatives of *a* and *b* chlorophylls, except where either component is definitely mentioned

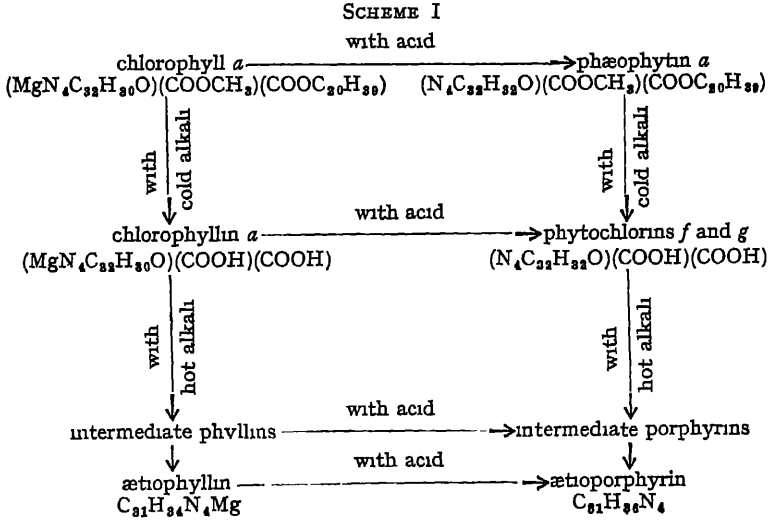
products is obtained by the removal of carboxyl groups, until in the final one no carboxyl group remains, and a substance devoid of oxygen results These substances are called phyllins, the final one in the case of both chlorophylls is ætiophyllin, possessing a formula $C_{31}H_{34}N_4Mg$ The phyllins are also acids containing magnesium bound in a complex way to nitrogen

The chlorophyllins and phyllins, like chlorophyll, lose their magnesium when acted upon by acids, with formation of bodies called porphyrins

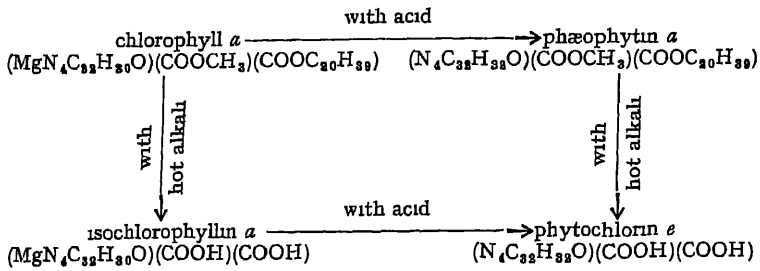
Special attention must be called to the action of *hot* alkalies on chlorophyll, which results in the production of substances with red fluorescence which are salts of acids called *isochlorophyllins*. Thus saponification with potassium hydroxide yields the potassium salts of isochlorophyllins When these are treated with acid, two substances are produced called phytochlorin *e* and phytorhodin *g*, the former a dissociation product of chlorophyll *a* and the latter the corresponding one of chlorophyll *b* Phytochlorin *e* is olive-green in colour, and phytorhodin *g* red The substances can be produced from chlorophyll by treating it first with acid and then treating the resulting phæophytin with hot alkali. These two substances, phytochlorin *e* and phytorhodin *g*, are of particular interest, because it was the formation of these two substances that led Willstatter to the discovery that there are two phæophytins, and hence that two chlorophylls are present in leaves.

The action of acids and alkalies on chlorophyll can be made clear by the following schemes The actions for chlorophyll *a* and its derivatives are shown Those of chlorophyll *b* and its derivatives are precisely similar

SCHEME I



SCHEME II



One characteristic of the saponification of chlorophyll remains to be mentioned When a mixture of chlorophyll *a* and chlorophyll *b* is treated with alkali, the green colour first changes to a deep brown. If the pure pigments are treated singly, chlorophyll *a* changes to yellow and chlorophyll *b* to red After a few minutes the colour changes back to green. Willstatter thinks this is possibly due to the presence in the chlorophyll molecule of a lactam ring CO—NH
| |
which is opened when the brown phase is produced, while another ring more stable towards alkalies is produced when the green colour returns This formation of the brown phase during saponification constitutes an important test for chlorophyll In alcoholic solution this brown phase is not given, and Willstatter supposes that chlorophyll then undergoes a change into an isomeric form in which the original lactam ring disappears, and another more alkali-stable one is formed

From the reactions of chlorophyll and its derivatives towards acids and alkalies, Willstatter was able to draw conclusions with regard to the constitution of the chlorophyll molecule, and to ascribe to chlorophyll *a* the formula

$$(C_{31}H_{20}N_3Mg)(CONH)(COOCH_3)(COOC_{20}H_{39})$$

and to chlorophyll *b* the formula

$$(C_{31}H_{27}N_3OMg)(CONH)(COOCH_3)(COOC_{20}H_{39})$$

In examining chlorophyll obtained from a number of different species, Willstatter and Stoll found that the proportion of phytol obtained from the different preparations of chlorophyll varied, while the theoretical yield should always be one-third of the weight of chlorophyll The plants in which the isolated pigment contained very little phytol were those in which it was found easy to obtain a crystalline preparation of a green pigment called crystalline chlorophyll. These findings were explained by Willstatter and Stoll as due to the presence in the leaves of an enzyme, chlorophyllase, continuing active in alcoholic media, which splits off the

phytyl group and in ethyl alcohol replaces it with an ethyl group, and in methyl alcohol by methyl. The substances so formed, termed chlorophyllides, are crystalline green compounds of which ethyl chlorophyllide *a* or crystalline chlorophyll *a* has the formula $(C_{32}H_{30}ON_4Mg)(COOCH_3)(COOC_2H_5)$, and methyl chlorophyllide *a* the formula $(C_{32}H_{30}ON_4Mg)(COOCH_3)_2$. Similar compounds are, of course, formed with chlorophyll *b* Plants yielding crystalline chlorophyll are hogweed (*Heracleum sphondylium*), hempnettle (*Galeopsis Tetrahit*), and hedge woundwort (*Stachys sylvatica*). Of plants poor in chlorophyllase, which should be used for obtaining true chlorophyll, Willstatter recommends the nettle (*Urtica sp*) as the most generally useful, as this is abundant, poor in enzymes and rich in chlorophyll It is interesting to note that the nettle was employed as a source of chlorophyll by Stokes in 1852.

When the chlorophyllides are treated with acids, magnesium is removed as it is from chlorophyll. The substances produced are called phæophorbides Thus methyl chlorophyllide *a* gives methyl phæophorbide *a* with the formula $(N_4C_{32}H_{32}O)(COOCH_3)(COOCH_3)$. This substance can also be obtained from phæophytin *a* by treatment with hydrochloric acid and methyl alcohol. Further treatment with concentrated hydrochloric acid splits off a methyl group with formation of phæphorbide *a* $(N_4C_{32}H_{32}O)(COOCH_3)(COOH)$, and subsequent treatment with alkali results in the removal of the second methyl group with the production of phytochlorin *e*, which can also be obtained direct from phæophytin by treatment with alkali, or, as already stated, by the action of acid on isochlorophyllin *a*

The methyl phæophorbides and these reactions in which they are concerned are important, as they are involved in the methods worked out by Willstatter for the quantitative estimation on the chlorophylls in fresh leaves

The methyl phæophorbides are best prepared from the methyl chlorophyllides, which can be obtained either from fresh leaves or from leaf powder If, for example, leaf powder of *Heracleum* be employed, this material is extracted with a mixture of four parts by volume of acetone and one part by volume of 80 per cent methyl alcohol, two litres of solvent being used for each kilogram of leaf powder, and the extraction allowed to proceed, with occasional shaking After filtering and further extraction of the material with acetone, the total filtrate is treated with talc and stirred with a glass spatula while about an equal quantity of water is added very slowly The methyl chlorophyllide then crystallises out and can be purified by washing successively with 50 per cent acetone, 90 per cent alcohol, petrol ether and ether

The methyl phæophorbides are easily obtained from the methyl chlorophyllides by dissolving the latter in a little pyridine, adding a large quantity of ether, and then strongly shaking the solution with 17 per cent hydrochloric acid This results in the formation

of the methyl phæophorbides, methyl phæophorbide *a* going to the acid solution, while the methyl phæophorbide *b* remains in the ether Further extractions of the ether with the acid solution remove the residue of the *a* substance from the ether, while the acid solution of the *a* component is freed from traces of the *b* substance by washing with ether.

This method of separation by means of hydrochloric acid is particularly worth noting It is due to Willstatter and Mieg (1906), who found that the various chlorophyll derivatives can be characterised by the way in which they distribute themselves between ether and hydrochloric acid, the concentration of the acid determining how much of a substance is extracted from it by ether. Thus, while only traces of phæophytin *a* are so extracted by an equal volume of 28 per cent hydrochloric acid, practically all is extracted by 32 per cent acid Only traces of phytochlorin *e* are extracted by 0·5 per cent acid, but almost all of it by 4 or 5 per cent. acid The " hydrochloric acid number " is the percentage content of that acid solution which on shaking removes two-thirds of the substance from an equal volume of an ethereal solution

The spectra of the chlorophylls are characteristic. In the case of chlorophyll *a* there is a strong absorption band in the red at the Fraunhofer line C There follow, in the yellow and green, four absorption bands decreasing in intensity, then a band of somewhat greater intensity in the blue, followed by a very strong absorption band in the indigo-blue, and the strongest absorption of all in the violet (end absorption). The spectrum of chlorophyll *b* is somewhat similar, but Willstätter, Stoll and Utzinger (1911) made out ten bands instead of eight, including the end absorption. There are two absorption bands in the red, a weaker one on the red side, and a stronger one just on the yellow side, of the C line ; then follow five weak bands in the yellow and green, a very strong absorption band in the blue, a strong absorption band in the indigo-blue and strong end absorption in the violet

It is interesting to note that the spectrum of a colloidal solution of chlorophyll differs somewhat in the position and number of the absorption bands from that of a true solution in ether or acetone. The main absorption, as before, is in the red and blue-violet end of the spectrum, but the spectrum resembles much more that of the living leaf, the absorption bands in each case being displaced towards the red end of the spectrum as compared with those of a solution of chlorophyll in acetone or ether (Tswett, 1910 , Herlitska, 1912*a*, *b*, *c* ; Willstatter and Stoll, 1913).

It is clearly of importance to know the condition of chlorophyll in the leaf Arnaud (1885) thought that the chlorophyll was held by capillary forces Reinke (1886) thought the chlorophyll was present in the chloroplast in the solid condition This opinion he based on the result of an experiment in which chlorophyll was dissolved in melted paraffin, which was then allowed to cool and set

solid. In the liquid state the solution fluoresces, but as the paraffin solidifies the fluorescence lessens considerably and is of about the same order as that of the leaf. Stern (1920, 1921) has pointed out, however, that the paraffin takes some time to solidify, and when it is completely solid the chlorophyll exhibits no fluorescence whatever, the fluorescence observed by Reinke being obviously due to the fact that the paraffin had not completely set solid.

Tswett (1901*a*) supposed that the pigment is adsorbed to the surface of the stroma of the chloroplast, while Palladin (1910) and Palladin and Stanewitsch (1910) suggested that the chlorophyll is combined with lipoid substances in the plastids. Iwanowski (1913) thought that the chlorophyll may be present as a fine suspension, and not in the colloidal condition, since the spectra of the living leaf and colloidal chlorophyll solutions are similar, but not identical. Herlitska (1912*b*), on the other hand, because of the agreement of the spectra of the living leaf and of a chlorophyll sol, an observation confirmed by Willstatter and Stoll, concluded that the chlorophyll in the two cases must be in a similar condition.

This is also the conclusion to which Willstatter and Stoll came. They put forward several arguments in favour of this view. In the first place, pure solvents such as acetone, ether and benzene do not extract the chlorophyll from dried leaves, but do so at once when a little water is added. It is supposed that the added water dissolves salts present in the dried leaves, that the salt solution precipitates the chlorophyll, which is then immediately soluble in ether. Colloidal solutions of chlorophyll behave in a similar way. Ether will only extract the pigment from a chlorophyll hydrosol after the addition of a little salt, such as calcium chloride or calcium nitrate.

In the second place, the chlorophyll in leaves is altered and made much more easily extractable by solvents by plunging the leaves in boiling water. The action of the latter is to bring about diffusion of the chlorophyll out of the plastids, while the colour of the leaf changes to a deeper green. At the same time the absorption bands in the spectrum of the leaf are displaced towards the violet, so that they occupy much the same position as those in a true solution of chlorophyll. This behaviour is at once explained on the view that the action of boiling water is to change the state of aggregation of the chlorophyll which changes from the sol condition to a true solution in the lipoid or waxy constituents of the plastids.

Finally, there is the argument based on the similarity of the spectra of the living leaf and of a chlorophyll sol.

This view has been called in question by Stern (1920, 1921), who regards the chlorophyll as present in the leaf in true solution. His conclusion is based largely on observations of the fluorescence of chlorophyll and green cells. Although it is not obvious, fluorescence is exhibited by green cells, as observed by Stokes (1852), Gümmler (1862), Hagenbach (1870, 1874), N. J. C. Muller (1873),

Reinke (1884*b*) and Tswett (1901*b*, 1911*b*) and confirmed by Stern himself for *Chlorella* and the green leaves of a number of different species The fluorescence of chloroplasts has been observed with the ultramicroscope by Gicklhorn (1914), Wilschke (1914) and Lloyd (1923*b*) The wave-length of the fluorescent light from green cells, as determined by different observers, is recorded in the following table:—

TABLE 6

WAVE-LENGTH OF FLUORESCENT LIGHT FROM GREEN CELLS

Plant	Observer	Range of wave-lengths.	Fluorescence maximum
Elder and Spinach . . .	Hagenbach	701–672	688
Spirogyra	Tswett	{660–640 {685–670	
Chlorella	Stern	705–664	681
Tradescantia	Stern	711–659	681

The two bands observed by Tswett with *Spirogyra* are probably due to the different fluorescence of the two chlorophylls They were observed with the aid of the fluorescence microscope Stern was unable to resolve the single band he observed into two, but considers that two bands would probably have been observed for the species he examined had he used that instrument

Stern next examined the fluorescence of chlorophyll in solutions and sols of various substances. He found a chlorophyll hydrosol does not fluoresce, nor does a chlorophyll sol when shaken with proteins, sugars or glycerol After shaking with fatty substances (triolein, lecithin, cholesterol, castor oil, etc), fluorescence is observed, however

The position of the fluorescent band in the spectrum is specially worthy of note With a solution of chlorophyll in alcohol the fluorescence maximum is at 654$\mu\mu$, with chlorophyll in lecithin it is at 677$\mu\mu$, and with *Chlorella* cells it is at 681$\mu\mu$.

From these various findings Stern concludes that the chlorophyll in the cell must be in true solution, and probably in solution in lecithin or some nearly allied lipoid substance While he has not disproved the possible existence of some chlorophyll in the colloidal condition in the chloroplast, there is no positive evidence in favour of it. Stern thinks, however, that the observation made by Buder (1913) on the very slight fluorescence of certain lower organisms suggests the possibility that colloidal or solid chlorophyll may be present in these Lloyd (1924) found, however, that when the blue-green algæ are heated enough to destroy the phycocyanin or phycoerythrin in them so that the chlorophyll in them is unmasked, the fluorescence spectrum of chlorophyll can be observed with the microspectroscope It lies between 650 and 700$\mu\mu$, and thus agrees well with the values found by Hagenbach, Tswett and Stern for plants belonging to other groups.

Stern's general conclusion with regard to the condition of chlorophyll in the chloroplast is therefore that the chloroplast is an emulsion or emulsoid with a chlorophyll-lipoid phase and an aqueous-protein phase. The droplets of lipoid are dispersed through the latter phase, but the chlorophyll itself is in true solution, that is, is molecularly dispersed, in the fatty droplets. This view, as will be shown in a later chapter, is used by Stern in developing his view of the mechanism of the photosynthetic process.

The Carotinoids

The yellow pigments of the plastids, unlike the chlorophylls, give non-fluorescent solutions These are stable in alkaline media, but easily acted upon by acids. A full account of the properties and reactions of the yellow pigments of the plastids, as well as of other carotinoids, will be found in the recent monograph by Palmer (1922).

Carotin —This pigment is identical with the yellow pigment of the carrot root, and is also present in etiolated leaves (cf. Coward, 1924*b*). It is not clear how far the colours of autumn leaves are due to carotin or xanthophyll or modifications of these pigments as they exist in green leaves, and the same remark holds with regard to the pigments of naturally yellow or variegated leaves and of yellow, orange and orange-red flowers (cf Tswett, 1908*b*, Goerrig, 1917, Palmer, 1922) The pigment in the tomato is a different substance (Millardet, 1876), isomeric with carotin, to which the name lycopin is given (Schunck, 1903).

Carotin is an unsaturated hydrocarbon of the formula $C_{40}H_{56}$, which crystallises in rhombohedra with a lustrous blue surface, appearing red in transmitted light Carotin is easily soluble in chloroform, benzene and carbon disulphide, it is soluble with difficulty in ether, petrol ether and in boiling methyl and ethyl alcohols, in cold methyl alcohol or ethyl alcohol it is almost insoluble Its solution in carbon disulphide has a red colour

Carotin undergoes oxidation if allowed to stand in air, in so doing it becomes bleached and increases in weight by 35 per cent in dry air and by 41 per cent in moist air It forms addition compounds with halogens In concentrated sulphuric acid it forms a deep blue solution

Xanthophyll —The xanthophylls possess the general formula of $C_{40}H_{56}O_2$ They also occur in etiolated leaves (Coward, 1924*b*) The crystals obtained by Willstatter's method are pleomorphic, often with a steel-blue lustre appearing yellow in transmitted light and red only where two or more cross one another Xanthophyll is insoluble in petrol ether and soluble with difficulty in methyl alcohol and carbon disulphide It is more readily soluble in ether and easily soluble in chloroform

Xanthophyll, like carotin, undergoes oxidation in air, bleaching

more quickly than carotin. It also forms addition compounds with halogens, and also gives a deep blue solution with sulphuric acid

Fucoxanthin.—This carotinoid, as isolated by Willstatter and Page (1914), crystallises out of methyl alcohol or acetone as dark red regular hexagons containing water or alcohol of crystallisation. As precipitated from ether with petrol ether it forms needles without any solvent of crystallisation. It possesses the empirical formula of $C_{40}H_{54}O_6$.

The pure crystals are completely insoluble in petrol ether, sparingly soluble in methyl alcohol and ether, fairly soluble in carbon disulphide and easily soluble in ethyl alcohol. The crystals do not readily oxidise, but the solutions readily bleach, absorbing oxygen to give a substance of the formula $C_{40}H_{54}O_{14}$ or $C_{40}H_{54}O_{16}$. Like carotin and xanthophyll, fucoxanthin dissolves in concentrated sulphuric acid to give a deep blue solution

The spectra of carotin, xanthophyll and fucoxanthin are all rather similar, showing two absorption bands in the neighbourhood of the F and G lines, together with some end absorption The actual position of the bands depends to a considerable extent on the solvent.

Phycoerythrin and Phycocyanin

There is little to be added concerning the red and blue plastid pigments to what has already been said in dealing with these pigments in general. As already indicated there, phycoerythrin is probably related to the proteins, but its exact composition is still a matter of doubt It has possibly never been prepared in an indubitably pure condition. Similar remarks apply to phycocyanin

Phycoerythrin is insoluble in pure water, but dissolves in a very weak solution of an alkali or of a neutral salt. According to Kylin the protein part can be separated from the pigment part of the molecule by heat, suitable concentration of acid or alkali, pepsin or trypsin After digestion of the protein, the pigment can be extracted with amyl alcohol

In neutral solution phycoerythrin is red, turning to red-violet in presence of acids and yellow on treatment with alkalies

The absorption spectrum of phycoerythrin is perhaps, from the point of view of photosynthetic considerations, its most important property It has been especially studied by Schutt (1888*a*, *b*) and Kylin (1910). It absorbs principally green rays, those which penetrate deep water Three well-marked absorption bands occur with maximum absorption at 569–565$\mu\mu$, 541–537$\mu\mu$, and 498–492$\mu\mu$ The absorption is thus well spread over the green region of the spectrum

Phycoerythrin exhibits deep orange fluorescence. Schutt found that only light of wave-lengths 600–486$\mu\mu$ could excite fluorescence in solutions of phycoerythrin, as, indeed, might be expected as the

absorption occurs over this interval; he expressed the opinion that the fluorescent light is probably of wave-length 590–560$\mu\mu$ Hanson (1909) found the fluorescence gave two well-marked bands, one at 655–630$\mu\mu$, the other at 600–570$\mu\mu$, the actual position of the bands varying, of course, with the solvent used.

Phycocyanin exhibits a red fluorescence, and Boresch (1921*d*) found that blue-green algæ containing this pigment fluoresce red, while those containing phycoerythrin exhibit yellow fluorescence Lloyd, who has made a special study of the fluorescence of the Cyanophyceæ by ultramicroscopy (1923*a*, *c*, *d*, 1924), concludes that the pigment is not held in the chromatophore, but is contained in extremely minute vesicles or vacuoles occurring in very great numbers, and so crowded that their conjoint fluorescence produces a flood of light which makes it extremely difficult to observe the structure of the cells.

The Extraction and Separation of the Plastid Pigments

Although much work was done during the nineteenth century on the preparation of the leaf pigments, it is now clear from the work of Willstätter that the earlier methods employed for the extraction and separation of the pigments were all faulty in one way or another It will not be necessary, therefore, to discuss this considerable quantity of work which preceded the investigations of Willstatter; it will be sufficient to give a short summary of the chief methods worked out by the latter and his co-workers.

1 *The Species chosen* —While the pigments in all leaves are the same, the pigments are not obtained with equal ease from all of them. If, therefore, it is simply a matter of obtaining the pigments without reference to any particular species, it is advisable to make a choice of material according to the end in view It will be recalled that some species are rich in an enzyme chlorophyllase which in alcoholic solution splits off phytol from chlorophyll with production of chlorophyllides (" crystalline chlorophyll ") If it is desired to obtain the true pigments, it is advisable to avoid such species, and for the preparation of the true chlorophylls Willstätter recommends the nettle (*Urtica sp*), as it is rich in chlorophyll and poor in chlorophyllase, it is also easily obtained in quantity. If, on the other hand, the chlorophyllides are wanted, species rich in chlorophyllase should be employed, such as *Galeopsis Tetrahit*.

2 *The Treatment of the Leaves* —While earlier investigators mostly extracted the leaf pigments by boiling fresh leaves in alcohol or other solvent, either with or without previous treatment, Willstatter advocates that the leaves should be first dried and ground to a fine powder This procedure has the advantage that it reduces the bulk of material and a smaller quantity of the extracting and other reagents can be used, while the leaf powder will

keep for a time, so that the extraction can be made to some extent independent of the season and habitat of the plants. It must be borne in mind, however, that the keeping qualities of leaf powder vary greatly among different species, while in some species chlorophyll is destroyed even by drying, as, for example, in the case of conifers and elder, unless special precautions are taken. Nettle-leaf powder can be prepared by spreading the leaves out on sheets of paper with another sheet of paper above them to prevent contamination with dust and undue exposure. They are then ground up and powdered, dried in an incubator for several days at 30° to 40° C, and finally transferred to a stoppered bottle, where the leaf pigments remain unchanged for a considerable time.

3. *The Extraction of the Pigments from the Leaves*.—As already pointed out, pure organic solvents will not extract the pigments from dry leaf powder, but such solvents readily do so if they contain a certain quantity of water. According to Willstatter, the most effective solvent is acetone containing 15 per cent. (by volume) of water, but he prefers to use 80 per cent. acetone, because less of the fats and waxes in the plastids are extracted by this more aqueous liquid [1].

4. *The Separation of the Chlorophyll from Carotinoids and Other Substances*.—The principles of the method of separation of the green and yellow pigments are as follows. The pigments are transferred from the acetone extract to petrol ether, from which accompanying colourless substances are washed away by treatment with aqueous acetone. The xanthophyll is then removed by addition of methyl alcohol, and the last traces of the latter and of acetone are then removed by washing with water. As the petrol ether becomes freed from these the chlorophyll is precipitated, and so is separated from the carotin, which remains in solution.

5. *The Separation of Chlorophyll a and Chlorophyll b*.—The principle involved in the separation of the two chlorophylls is that if a mixture of the two is treated with petrol ether and 90 per cent. methyl alcohol together, the chlorophyll *a* prefers the petrol ether and the chlorophyll *b* the methyl alcohol. The separation is, however, far from complete, and it requires about sixteen successive extractions of the petrol ether with methyl alcohol to free the former from chlorophyll *b*. The purification of the methyl alcohol solution from chlorophyll *a* is equally tedious. Those proposing to separate the two chlorophylls should not fail to consult Willstatter and Stoll (1913), where the details of the method of fractionation are given.

6. *Purification of Xanthophyll*.—The solution of xanthophyll in methyl alcohol, obtained as previously indicated, contains a quantity of chlorophyll *b*. This is easily removed by adding ether to the

[1] For practical details of the extraction and preparation of the assimilatory pigments reference should be made to Willstätter and Stoll (1913).

methyl alcohol and then shaking with some concentrated solution of potassium hydroxide in methyl alcohol, whereby the chlorophyll is saponified to chlorophyllin, which is then removed by washing with water The ethereal solution of xanthophyll is then dried with sodium sulphate, evaporated down to a very small bulk and treated with methyl alcohol On removing the ether completely by re-evaporation, and then filtering the hot solution, xanthophyll crystallises out on cooling. Water may be added to make the separation of the xanthophyll complete.

7. *Purification of Carotin* —The carotin is obtained by evaporating *in vacuo* the solution obtained as previously described. The residue is treated with 90 per cent. alcohol, from which the carotin crystallises out Purification is effected by dissolving the crystals in petrol ether and filtering from any impurity, and the process may be repeated with a mixture of two parts of petrol ether and one part of alcohol

8. *Preparation of Fucoxanthin from Brown Algæ* —The principles involved in the method of preparation of this pigment as worked out by Willstatter and Page (1914) are as follows. The fresh thallus is extracted with 40 per cent acetone and the extract pressed out by means of an hydraulic press and discarded. The residue is then pounded up and extracted with 85 per cent. acetone So much water is then added to the extract that the chlorophyll is precipitated, while the greater part of the fucoxanthin remains in solution. The fucoxanthin is then extracted from the liquor by shaking with a mixture of three parts of petrol ether and one part of ether Acetone is removed from the ethereal solution by washing with water On shaking the ethereal solution with 70 per cent methyl alcohol saturated with petrol ether, the fucoxanthin and xanthophyll pass into the methyl alcoholic layer. The xanthophyll is removed by shaking with an equal volume of a mixture of five parts of petrol ether and one of ether As the latter liquid contains a considerable quantity of fucoxanthin, it is evaporated *in vacuo* to a small bulk, diluted with ether and extracted with 70 per cent methyl alcohol, the methyl alcoholic extract containing the fucoxanthin being washed with the ether-petrol ether mixture The fucoxanthin in the methyl alcohol extracts is then transferred to ether by the addition of a large quantity of that solvent The filtered ethereal solution is evaporated at a low temperature to a syrup, and the fucoxanthin precipitated by addition of low boiling-point petrol ether Purification can be effected by re-crystallisation from methyl alcohol, when crystals are obtained containing three molecules of methyl alcohol of crystallisation which can be removed *in vacuo*, or solvent free crystals can be obtained by re-precipitation from ether with low boiling-point petrol ether.

Quantitative Estimation of Chlorophyll in an Extract

Willstatter has worked out a simple method for estimating the quantity of chlorophyll ($a+b$) in an extract For this purpose 10 c.c. of the acetone or alcohol extract is diluted to 100 c c. with ether, and 10 c c. of this poured into a separating funnel and diluted with a further 40 c c. of ether. If the original extract contained acetone, this is completely removed by adding a little methyl alcohol and washing the solution thoroughly with water, which is then removed. To the ether or ether-diluted alcohol extract 4 or 5 c c. of methyl alcoholic potash are added, the mixture shaken, and the brown phase will appear (cf p 26). After the reappearance of the green colour with the formation of the potassium salts of chlorophyllins, water is slowly added while the separating funnel is gently rotated, and the aqueous chlorophyllin solution run into a 200 c.c measuring flask. The ether solution of the yellow pigments is washed with a little more water to extract the chlorophyllins completely, and the aqueous layer added to that in the flask. The whole is then made up with alcohol to 200 c c.

The chlorophyllin solution is then estimated in a colorimeter by comparison with a standard solution made up from a known quantity of pure chlorophyll which has been similarly transformed to the chlorophyllin salts.

Quantitative Estimation of Pigments in Fresh Leaves

While for some plant physiological work the quantitative estimation of chlorophyll in an extract may be sufficient, it is obvious that the estimation of the quantities of the pigments in fresh leaves is likely to have a very much greater value Methods for making such estimations have also been worked out by Willstatter; the principles underlying the methods will be indicated below, but for the practical details reference must be made to Willstatter and Stoll (1913)

1. *The Extraction of the Pigments* —A preliminary treatment of the ground leaf material with 40 per cent acetone, followed by 30 per cent. acetone, softens the leaf material, removes acids, inhibits enzyme action, but removes no chlorophyll The pigments are then extracted from the residue with pure acetone, successive extractions being made until all the pigments are removed Towards the end of the extraction 5 to 10 per cent. of water is added to the acetone The pigments are then transferred to ether, and acetone removed by washing with water After drying with anhydrous sodium sulphite the extract is divided into two equal parts, one of which is used for determination of the chlorophylls, the other for estimation of the carotinoids

2. *Separation of Chlorophyll* a *and Chlorophyll* b —The ethereal solution is treated with hydrochloric acid in order to convert the

chlorophylls to the phæophytins The ether is evaporated off and the residue dissolved in a very little pyridine, and, while being heated on a steam bath, is treated with boiling methyl alcoholic potash with production of isochlorophyllins (cf. p. 25). The liquid is now acidified with 20 per cent hydrochloric acid, which converts the isochlorophyllins to phytochlorin *e* and phytorhodin *g*. These derivatives of chlorophyll *a* and chlorophyll *b* respectively are now extracted from the ether, first with 12 per cent. hydrochloric acid and then with 20 per cent acid, until no more is removed by the acid The ethereal layer then only contains carotin and other impurities The acid extract is now neutralised with ammonia and the pigment derivatives transferred back to ether, from which, after washing with about 0·6 per cent hydrochloric acid, the phytochlorin *e* is extracted with 3 per cent hydrochloric acid, and finally with 5 per cent. acid, the extracts with this stronger acid being fractioned by neutralising, transferring to ether, and extracting this with 3 per cent. hydrochloric acid The phytochlorin *e* solution so obtained is used for determining chlorophyll *a* The remaining liquid, containing the phytorhodin *g*, is treated several times with 12 per cent hydrochloric acid to extract the phytorhodin *g*

3. *The Separation of the Xanthophyll and Carotin* —The ether extract from fresh leaves is saponified with concentrated methyl alcoholic potash, and the chlorophyllin salts produced from the chlorophylls removed by washing with water The two yellow pigments are now separated by making use of the fact that in presence of methyl alcohol and petrol ether xanthophyll goes to the methyl alcohol and carotin to petrol ether. The carotinoids in ethereal solution are accordingly first washed with water and methyl alcoholic potash, and then more water to remove impurities, they are then transferred to petrol ether after evaporating off the ether; the xanthophyll is then removed by repeated extractions with methyl alcohol The xanthophyll is transferred to ether by adding the latter to the methyl alcohol, slowly adding water and separating the aqueous methyl alcoholic layer. The methyl alcohol is completely removed by further washings with water, the solution filtered, cleared by addition of a few drops of absolute alcohol and made up with ether to a standard volume. The petrol ether solution of carotin is similarly treated

4 *The Method of Estimation* —The determinations of the four pigments are made colorimetrically by comparison with standard solutions Those for the estimation of the chlorophylls are prepared by the saponification of a mixture of the methyl phæophorbides containing methyl phæophorbide *a* and methyl phæophorbide *b* in the molecular proportion of 3 : 1. The relative quantities would, of course, have to be altered in the case of algæ where the chlorophylls occur in a ratio different from 3 : 1. The phytochlorin *e* and phytorhodin *g* are then extracted with 3 per cent and 12 per cent.

hydrochloric acid respectively, and these extracts form the standard solutions.

The standard solutions of carotin and xanthophyll are prepared by dissolving a known weight of these substances direct in petrol ether and ether respectively Instead of using solutions of the pigments it is possible to employ a solution of potassium dichromate as a standard, and Willstatter and Stoll give a table of thicknesses of aqueous solutions of this salt, and of the solutions of the yellow pigments, which correspond to one another in intensity.

A method of determining carotin quantitatively by means of the spectrophotometer has recently been described by Schertz (1923), who considers the method superior to the colorimetric one both on account of accuracy and ease of reading the instrument It seems possible that the same method might be used for the determination of the other leaf pigments

Variations in the Quantity of the Leaf Pigments in Fresh Leaves

By means of the methods described in the preceding section, Willstatter and Stoll have determined the quantities of the four leaf pigments in the leaves of a number of different species growing under a variety of conditions. The chief results of their investigation are summarised below

1 *The Total Content of Green and Yellow Pigments*—The chlorophyll content of leaves appears to vary from 0 6 to 1·2 per cent of the total dry weight, the usual value being about 0 8 per cent, of which three-quarters is chlorophyll *a* and the rest chlorophyll *b*. Considerably greater variations were observed among leaves of the same plant than between the mean values of the chlorophyll content of leaves from different plants. In relation to the dry weight, shade leaves are relatively richer in chlorophyll than sun leaves, but as shade leaves are often very thin, this relation does not hold relative to the leaf surface.

The total content of carotinoids varies from 0·1 to 0 2 per cent of the dry weight, the carotin representing from 0 03 to 0 08 per cent, and the xanthophyll from 0 07 to 0 12 per cent. of the dry weight. Shade leaves do not contain a higher content of yellow pigments in relation to the dry weight

The time of day is without influence either on the total quantities of pigment or on the ratio of chlorophyll *a* to chlorophyll *b*, or on the ratio of xanthophyll to carotin This must be regarded as an important fact in regard to the mechanism of the photosynthetic processes.

2. *Variations in the Proportions of the Two Chlorophylls.*—The mean ratio of chlorophyll *a* to chlorophyll *b* is 2 85, the greatest divergence from this value being about 0 75 The variations which exist appear to be brought about by external conditions Thus

plants such as *Sambucus*, growing in shade, exhibit abnormal chlorophyll ratios, whereas true shade plants such as *Fagus sylvatica* possess a normal chlorophyll content

On the whole, shade leaves contain relatively less chlorophyll *a* than plants growing in more sunny habitats The average ratio of chlorophyll *a* to chlorophyll *b* for shade plants is 2 61, while excluding shade plants the ratio is 2 93.

3 *Variations in the Proportion of Xanthophyll to Carotin.*—The mean value of the ratio of carotin to xanthophyll is 0 546 ±0 2. In regard to this ratio shade leaves show a wider divergence from the normal than they do in regard to the chlorophylls Thus the average ratio of carotin to xanthophyll in shade leaves is 0 421 ±0·1 as compared with 0 603 ±0·1 for normal leaves

4. *The Ratio of Green to Yellow Pigments* —The average molecular ratio of chlorophyll to carotinoids is 3 56, the ratio being 3 07 for sun leaves and 4 68 for shade leaves In plants well suited for growth in the shade still higher values are found , thus in the beech a value for the ratio of 6·02 was recorded by Willstätter and Stoll On the other hand, shade leaves of *Platanus acerifolia* examined by these workers gave a low value, namely 3 31.

Although in shade leaves chlorophyll *a* and xanthophyll are present in relatively greater amount than chlorophyll *b* and carotin respectively as compared with normal leaves, no simple relation could be found between the ratio of the chlorophylls and the ratio of the carotinoids

CHAPTER IV

THE DEMONSTRATION OF PHOTOSYNTHESIS

In the first chapter it was shown that the complete photosynthetic process consists of the absorption of carbon dioxide by the green parts of plants in light, with the consequent formation of carbohydrates and evolution of oxygen. That carbon dioxide is indeed absorbed by the assimilating organs while oxygen is evolved and carbohydrates are formed, is easily demonstrated.

The Absorption of Carbon Dioxide

That the absorption of carbon by the plant takes place exclusively by the intake of carbon dioxide by the leaves and other green organs of plants, is made very clear by the growth of plants in sand or water free from organic material and containing only inorganic salts That the leaves do indeed absorb carbon dioxide under suitable external conditions of light and temperature, can be easily demonstrated by the method employed by Pfeffer (1871) and other workers subsequently (see *e g.* Holle, 1877). Pfeffer's apparatus, frequently figured in plant physiological text-books, consists of a vessel open at the lower end and about 36 cms. long, of which the lower part consists of a graduated tube 26 cms long and the upper part of a bulb about 1 5 cms in diameter, terminating above in a narrow tube which can be connected to an aspirator or pump by means of rubber tubing. The vessel has a content of about 120 c c (Fig. 3). A leaf is inserted in the bulb, this can be accomplished easily by rolling up the leaf and pushing it up the tube by a glass rod, when the leaf reaches the wider part of the tube it unrolls, and after the experiment it can be removed by means of a thin wire which is attached to the leaf-stalk The vessel is then placed with the lower end of the graduated tube in a vessel of mercury, and mercury is then drawn up the cylindrical tube to a height of a few centimetres The tube connected to the upper end of the vessel is then closed by means of a clip and a known quantity of carbon dioxide introduced into the vessel The level of the mercury in the graduated tube is noted The apparatus is then exposed to light for a requisite length of time depending on

the intensity of the light (about 5 hours in bright diffuse light), after which the level of the mercury is again noted and the leaf is removed. The quantity of carbon dioxide present in the vessel is now determined by introducing about 0 02 c.c. of concentrated potassium hydroxide and again observing the level of the mercury. From the decrease in volume so determined, the quantity of carbon dioxide removed by the leaf is at once obtained, due notice being taken of temperature and pressure. In this way the absorption of carbon dioxide by leaves is easily demonstrated Practical details for performing this experiment with this apparatus and various modifications of it are given by Darwin and Acton (1901)

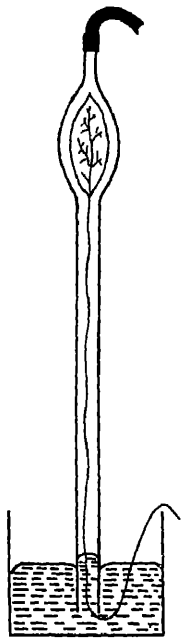


FIG 3 — Eudiometer for demonstrating and measuring the absorption of carbon dioxide and evolution of oxygen by assimilating leaves (After Pfeffer)

THE PRODUCTION OF OXYGEN

A number of ingenious methods for demonstrating easily the production of oxygen in photosynthesis have been devised

1 *The Bubbling Method.*—This is the best known method of observing the production of oxygen in assimilating plants It dates back to Dutrochet (1837), and still maintains its popularity in botanical laboratories It is, however, only applicable to water plants.

A piece of water plant such as *Elodea* or *Potamogeton*, or a green fresh-water alga, when placed in water and exposed to sunlight, evolves a constant stream of bubbles. If these bubbles are collected by placing an inverted funnel over the plant, and an inverted test-tube of water over the funnel, the gas can be collected and examined chemically, when it will be found that at least a considerable portion of it consists of oxygen

2 *The Indigo Method* —When an aqueous solution of indigo-carmine (indigotin) is reduced by sodium hydrogen hyposulphite, $NaHSO_2$, the blue colour disappears If a vessel containing a shoot of a water plant such as *Elodea* or *Potamogeton* is immersed in a solution of decolorised indigo-carmine contained in a closed vessel, exposure to light at suitable temperature will result in the reappearance of the blue colour of the indigotin around the green leaves owing to oxidation brought about by the oxygen evolved in assimilation According to Palladin (1911, 1918) nigrosin can be used instead of indigotin The method is, of course, chiefly applicable to water plants, but can be used with land plants

3 *The Blood Method*—This method, due to Hoppe-Seyler (1879) and Engelmann (1888*a*), consists in immersing a leaf or other chlorophyll-containing organ in defibrinated ox blood in the venous condition, and exposing to light. The evolution of oxygen by the leaf produces the red colour of oxyhæmoglobin around the leaf The method is thus the same in principle as the indigo method The method may be made more delicate by observing the change in the spectrum of the blood with the appearance of the oxyhæmoglobin.

4 *The Phosphorus Method*—Boussingault (1869) introduced a method in which the evolution of oxygen is made obvious by the oxidation of phosphorus into phosphorus pentoxide A vessel containing shoots of a water plant such as *Potamogeton* is filled with water and part of the latter replaced with hydrogen. The mouth of the vessel is then closed by a cork to which is attached by a pin a piece of phosphorus which projects into the atmosphere of hydrogen. The vessel is placed in the dark for a time, and on bringing into the light evolution of oxygen can be recognised at once by the formation of white fumes of phosphorus pentoxide

5. *Engelmann's Bacteria Method*—This method (Engelmann, 1881, 1883, 1886, 1887, 1894, Beijerinck, 1890) is perhaps the most sensitive of the methods for demonstrating the evolution of oxygen It depends on the sensitiveness of certain bacteria, such as *Pseudomonas fluorescens*, to the presence of oxygen A thread of a filamentous alga, for example, is mounted in water containing the bacteria, covered with a glass slip, and the edge of the slip sealed to prevent the entrance of air If the preparation is kept in the dark the movements of the bacteria gradually stop as the supply of oxygen is exhausted. On exposure to light the movements of the bacteria recommence round the assimilating filament owing to the presence of oxygen which is evolved there The method can be used equally well with the leaves of higher water plants

6. *Beijerinck's Phosphorescent Bacteria Method*—Beijerinck (1901) made similar observations with phosphorescent bacteria which require oxygen for their phosphorescence. When brought together with a green alga it was found that the bacteria only phosphoresce when the alga has been exposed to light, the necessary oxygen for this being evolved from the alga during assimilation (see also Molisch, 1904)

7 *Pfeffer's Method*—The methods described above are all more applicable for use with water plants than with land plants, although the use of the latter is not always excluded But to show the evolution of oxygen by the leaves of land plants when exposed to sunlight Pfeffer's method, described already in regard to the demonstration of the absorption of carbon dioxide, can be used also to demonstrate the evolution of oxygen, the amount of this present at the end of the experiment being determined by observing the diminution in

volume of the gases in the vessel after the introduction of a few cubic centimetres of a solution of pyrogallol.

The Production of Carbohydrates

The demonstration of the formation of carbohydrates in leaves or other assimilating organs is very simple in the case of those leaves which form starch. In leaves which do not produce this substance the demonstration of carbohydrate production is not so easy

The demonstration of the production of starch is carried out by means of Sachs's Iodine Method (Sachs, 1884) Leaves are taken from a plant which has been kept in the dark for some time; and from the same plant after it has been subsequently exposed to sunlight for some time The leaves are decolorised by boiling for a few minutes in water and then immersing in warm alcohol After removal of the chlorophyll in this way the leaves are placed in a solution of iodine in water. If starch is present it is stained deep violet by the iodine, and so can be recognised by naked-eye inspection. If the times of exposure to dark and light have been sufficient it is observed that after a period in the dark the leaves contain no starch, which, however, reappears after sufficient exposure to the light.

With filamentous algæ or very thin leaves the starch so stained can be observed under the microscope. With thicker leaves sections can be used, or the leaves can be rendered transparent by immersion in a concentrated solution of chloral hydrate to which the solution of iodine is added (Schimper, 1885*b*)

In many plants, however, starch is not formed, and in others it is not formed in any quantity Even in plants where starch is abundant it is exceedingly improbable that it is the first carbohydrate to be formed in the leaves That sugars are formed in leaves during illumination may be demonstrated by gathering leaves of, for example, *Allium Cepa*, before and after a period of illumination, and determining the power of the expressed sap to reduce Fehling's solution It is found that the reducing power of the juice increases considerably as a result of exposure to light. That the reducing substances produced are actually sugars is confirmed by the preparation of the phenyl osazone of glucose from the expressed sap, while crystals of sucrose were actually isolated from leaves of the vine by Kayser as long ago as 1883

The experimental details of the demonstration of these fundamental facts of photosynthesis need not be described in this place. Reference may be made to easily accessible practical text-books such as those of Detmer (1898), Darwin and Acton (1901), and Kolkwitz (1914), in which practical details for performing these experiments are given.

CHAPTER V

THE MEASUREMENT OF PHOTOSYNTHESIS

Introductory Remarks

It is clearly of great importance for obtaining an understanding of the photosynthetic processes that reliable methods should be available for determining the rate of assimilation under natural and controlled conditions A number of methods have been devised by different workers. These methods fall into three groups depending respectively on the determination of (1) the absorption of carbon dioxide, (2) the evolution of oxygen, and (3) the formation of carbohydrate. If the general equation

$$6CO_2+6H_2O=C_6H_{12}O_6+6O_2$$

represents correctly the whole of the assimilatory process, these various methods will all give the same result We know, however, that in some plants sucrose and starch are formed as well as hexose sugars, so that equations such as

$$12CO_2+11H_2O=C_{12}H_{22}O_{11}+12O_2$$

and

$$6nCO_2+5nH_2O=(C_6H_{10}O_5)_n+6nO_2$$

would more correctly represent the whole process In such cases it is clear, provided the whole of the carbon absorbed is used in assimilation, that the measurement of the rate of absorption of carbon dioxide and the rate of evolution of oxygen will both give measures of the carbon assimilated The amount of carbohydrate will, however, not necessarily bear a constant relation to the carbon assimilated, but will vary with the proportions of the various carbohydrates that make up the whole. A determination of the actual gain in *carbon* should, on the other hand, give results exactly similar to those obtained by measuring carbon dioxide intake or oxygen evolution

It is generally assumed that only carbohydrates are produced in photosynthesis. If, on the other hand, other substances such as organic acids or fats should be formed, either as by-products of the action or as storage products, the values of assimilation given by measuring carbon dioxide absorption and oxygen evolution are

not likely to correspond, as only exceptionally will the volume of carbon dioxide absorbed equal that of oxygen evolved, and it will only bear a constant ratio to it if the ratios of the various products are constant. If photosynthesis of proteins and other nitrogen compounds also takes place in the leaf (cf Chapter XI) the matter is even more complicated

One difficulty is common to all the methods. This results from the complication introduced by respiration. It is a generally accepted fact that every living cell respires, and that green cells respire like all other living cells has been abundantly shown by experiments with such material in the dark, in which the absorption of oxygen and evolution of carbon dioxide has been consistently observed

There appears to be no *a priori* reason why it should be accepted as a fact that assimilation and respiration proceed independently in the same cell, and, indeed, it would rather appear difficult to accept, without evidence, the view that both a reduction process and an oxidation process should proceed together in this way On the other hand, it is difficult to suppose that respiration, which proceeds in leaves in the dark, should immediately cease when the leaves are brought into the light. And Bernard (1878) showed that treatment with chloroform will inhibit the assimilatory process while respiration will still proceed in leaves. More direct evidence of contemporaneous photosynthesis and respiration is derived from the fact that during assimilation in green cells, movement of protoplasm and also growth take place, two phenomena that are bound up with respiration (cf Benecke, 1924)

In a green assimilating cell, therefore, the two processes of assimilation and respiration may be assumed to proceed simultaneously, the one involving absorption of carbon dioxide, manufacture of carbohydrates and evolution of oxygen, the other the evolution of carbon dioxide, the breaking down and disappearance of carbohydrates and absorption of oxygen The carbon dioxide absorbed from outside will therefore be less than that actually used in photosynthesis by the amount given out in respiration, which will be utilised in photosynthesis before it can diffuse out of the assimilating cell. The absorption of carbon dioxide actually measured gives, therefore, the value of the "apparent assimilation", to obtain the value of the "true assimilation" the carbon dioxide evolved in the same time must be added to the value of the assimilation actually measured.

Similarly the oxygen actually evolved from an assimilating plant will also only give a measure of the apparent assimilation, for part of the oxygen formed in photosynthesis will be directly utilised in respiration. To obtain the value of the true assimilation the oxygen absorbed in respiration must be added A similar correction must be made when the assimilation is measured by determining the increase in carbohydrate

It is only to be expected that the same methods of measuring photosynthesis will not be suitable for land plants and water plants alike, each group presenting characteristics which render special arrangements necessary In experiments with land plants carbon dioxide must be supplied in gaseous form, and it is a comparatively simple matter to provide such plant material with an external atmosphere containing any proportion of carbon dioxide from 0 to 100 per cent. The supply of carbon dioxide to submerged water plants requires more consideration. The concentration of carbon dioxide in water in equilibrium with the atmosphere depends on the partial pressure of the gas in the atmosphere and on the temperature At 15° C the concentration of the gas in water is about the same as in the air with which the water is in equilibrium, but the temperature has a very considerable influence on the quantity of carbon dioxide which water will take up, water at 20° C only absorbing about half that it absorbs at 0° C It is now supposed that the greater part of the carbon dioxide absorbed remains as such in the water · in a 0 1 volume per cent solution of carbon dioxide in water, only 8 per cent. of the carbon dioxide combines with water to form carbonic acid H_2CO_3, which is almost completely dissociated into H· and HCO'_3. The quantity further dissociated into H and CO''_3 is negligible.

Instead of dissolving carbon dioxide directly in water, a solution of a bicarbonate can be employed as a source of carbon dioxide The carbon dioxide concentration of water containing a bicarbonate can be greater than that corresponding to the partial pressure of carbon dioxide in the atmosphere When, for example, potassium bicarbonate is dissolved in water part is dissociated into K and HCO'_3, and only a negligible amount is further dissociated into H· and CO''_3 With the H ions of the water present there is thus a certain small quantity of carbonic acid present which will mostly be transformed to carbon dioxide and water, and the formation of carbonic acid and carbon dioxide will proceed to the point at which the system is in equilibrium In the case of sodium bicarbonate about 2 68 per cent of the bicarbonate is transformed in this way (McCoy, 1903).

According to Angelstein (1911), potassium bicarbonate is more suitable as a source of carbon dioxide for plants than sodium bicarbonate, on account of the somewhat toxic action of the latter Potassium bicarbonate is toxic in high concentrations , a solution containing 1 per cent of potassium bicarbonate is a satisfactory medium for *Elodea*, but *Fontinalis* is adversely affected by a concentration of 0 64 per cent

It is most generally agreed that carbon dioxide, and perhaps carbonic acid, are the only sources of assimilatory carbon (cf Nathansohn, 1910 , Benecke, 1921). Nevertheless, it has recently been suggested that the HCO'_3 ion is absorbed and utilised in assimilation Ruttner (1921) has followed the change in electrical

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conductivity of calcium carbonate solutions containing assimilating water plants, and concludes that the HCO'_3 ions are absorbed and utilised in assimilation, OH′ ions being excreted The interpretation of the experimental results is, however, not very clear.

In any case the withdrawal of carbon dioxide from water by assimilating plants must render the water more alkaline whether HCO'_3 ions are absorbed directly and replaced by OH′ ions, or whether the carbon dioxide is withdrawn as such with the result that the equilibrium is disturbed and the quantity of H· and HCO'_3 ions present lessened on this account. The slight variations in the hydrogen ion concentration of shallow waters are attributed to the photosynthetic activity of the plants present (Saunders, 1920), while it was found that during the year 1922 the hydrogen ion concentration of the sea at Plymouth rose to a maximum in May, fell to a well-defined minimum in July and rose again to a second maximum in August, the maxima corresponding in a general way to the maximum photosynthetic activity of the diatoms of the sea (Atkins, 1923; see also 1922 and 1924). In running brooks, however, there is little change in the hydrogen ion concentration during night and day, the vegetation of the beds of streams having no appreciable effect on the hydrogen ion concentration because of the relatively large total volume of water involved (Duval and Dumaraud, 1923). It is true that after leaving the source of the stream the water becomes slightly more alkaline, but this is ascribed to liberation of carbon dioxide dissolved in the water, owing to the agitation of the water brought about by the assimilating plants.

It will be seen, then, that two different ways of classifying the methods of measuring assimilation are possible, one based on the type of plant, the other on the principle involved in the method It is immaterial which system is followed, but in the outline of the various methods given below, the latter system is adopted.

Methods based on the Intake of Carbon Dioxide

1. *Eudiometric Method* —The simplest method of measuring the assimilation of an aerial plant organ is by the eudiometric method described in the preceding chapter The characteristic of the method is that the assimilating organ is kept in a closed vessel and the change in the concentration of the carbon dioxide in the vessel is determined after a known lapse of time Pfeffer's apparatus, described on a previous page (p 40), was used by its designer to obtain quantitative data, but the fact that the absorption of carbon dioxide renders the concentration of the latter a changing quantity throughout the experiment introduces a complication Also, what is a more serious drawback to the method, if the experiment is of any but short duration, transpiration from the leaf into

a closed space disturbs the water relations of the leaf or other assimilating organ.

Various modifications of this apparatus are possible and some have been especially designed for work on gaseous exchange in plants. Thus, as well as the ordinary gas analysis apparatus of Winkler and Hempel and of Haldane, mention may be made of an apparatus designed by Osterhout (1918*a*), and also of the micro-eudiometer of Timiriazeff (1885*a*) and the capillary eudiometer of Bonnier and Mangin (see Aubert, 1891, Thoday, 1913*a*), which have been designed specially for determining small quantities of oxygen and carbon dioxide such as frequently have to be measured in work on plant respiration and assimilation For details of these pieces of apparatus reference may be made to the works cited. For a description of other methods of analysis of carbon dioxide and oxygen, including the apparatus of Hempel, Winkler, Reiset, Krogh and Thunberg, reference may be made to Grafe's practical text-book (1914), in which eudiometric methods of determining photosynthesis are dealt with in some detail

Whatever form of apparatus is used, the value of assimilation actually obtained by experiment must be corrected for respiration For this purpose the respiration must be determined by means of an experiment made in the dark.

2 *Continuous Current Method*.—It has been noted above that eudiometric methods of measuring photosynthesis are open to the objection that the composition of the gas in the experimental chamber is not constant during the course of an experiment, and that the water relations of the leaf are liable to become disturbed These difficulties can be very largely eliminated by the use of a method which appears to have been first employed by Kreusler (1885), and which has subsequently been modified and improved by F F Blackman (1895*a*), Giltay (1898), Matthaei (1904), Blackman and Matthaei (1905), Brown and Escombe (1905*a*), Willstatter and Stoll (1918), and Spoehr and McGee (1923, 1924*a*). The principle of this method is that the assimilating plant material is contained, not in a completely closed chamber, but in one through which passes a constant stream of gas containing a known proportion of carbon dioxide. After leaving the plant chamber the gas passes through tubes in which the carbon dioxide is absorbed and so determined The original concentration of carbon dioxide and the quantity of gas which has passed through the apparatus being known, the carbon dioxide absorbed by the plant material is at once found by difference. In this way the concentration of carbon dioxide in the gas external to the assimilating material is kept approximately constant and known during the course of an experiment, while water transpired from the leaf is removed in the gas current Wilting of the leaf is prevented by keeping its stalk in a small vessel of water contained in the chamber

The method as employed by different workers exhibits note-

worthy variations, especially in regard to the form of the leaf chamber and the method of determining carbon dioxide. Thus, in the work of Blackman and Matthaei a single leaf was employed in each experiment, and this leaf was contained in a flat chamber with glass faces and wood sides (Fig. 4) In Willstatter and Stoll's work a number of leaves were contained in a flat glass dish provided with a flat glass cover, the laminæ of the leaves being supported by means of a silver grid through the meshes of which the stalks passed, the lower ends of the stalks dipping in water covering the floor of the dish.

In Kreusler's experiments the carbon dioxide, after passing through the assimilation chamber, was absorbed by barium hydroxide. Brown and Escombe measured the carbon dioxide content of the gas after passing over the leaf, and also of a branch-stream of the same gas which did not pass through the assimilation chamber, by leading the gas into Reiset towers containing sodium hydroxide. The quantity of gas in each stream was measured by means of a gas meter. Blackman and Matthaei used a single current of gas containing the required concentration of carbon dioxide, and this current, by means of an automatic device, was divided into two exactly equal half-currents The carbon dioxide in the two currents was determined by absorption with baryta water and subsequent titration. One of the currents was therefore led directly through a Pettenkofer tube, the other first through the leaf chamber.

Willstätter and Stoll allowed the gas stream of known composition to issue at constant velocity from a cylinder After the gas had passed through the assimilation chamber the carbon dioxide was absorbed by soda-lime and determined by the gain in weight of the latter.

In Spoehr's experiments the carbon dioxide was also absorbed by barium hydroxide solution, but the estimation was made by determining the change in electrical conductivity of the baryta water. Previous determinations of the electrical conductivity of solutions of barium hydroxide which had absorbed known quantities of carbon dioxide, rendered a determination of the carbon dioxide in the gas stream a very simple matter.

Nothing more than an outline of the principle involved in the continuous current method and its various modifications has been attempted. For details of the experimental arrangements of the various workers, the original papers cited above must be consulted

There can be little doubt that the continuous current method is the most reliable of all the methods that have been evolved for measuring photosynthesis. Complications are reduced to a minimum, and while the plant material used is not under natural conditions, the conditions of the experiment are more exactly definable than in any other method

A modification of the method for use with water plants was

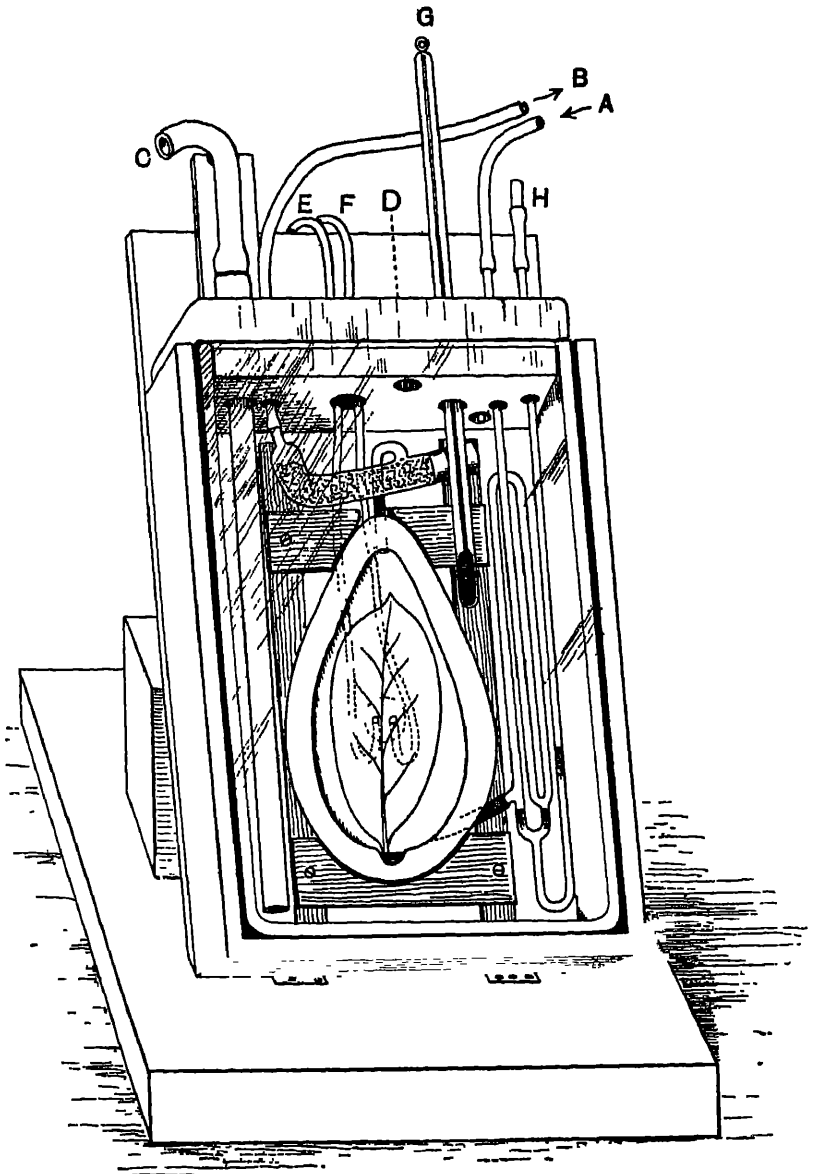

FIG 4.—Leaf chamber used in the determination of photosynthesis by the continuous gas stream method (Reproduced by permission of Dr F F Blackman and the Council of the Royal Society)

devised by Blackman and Smith (1911*a*). Instead of the continuous gas stream, a stream of water charged with carbon dioxide was employed. This flows through the assimilation chamber in

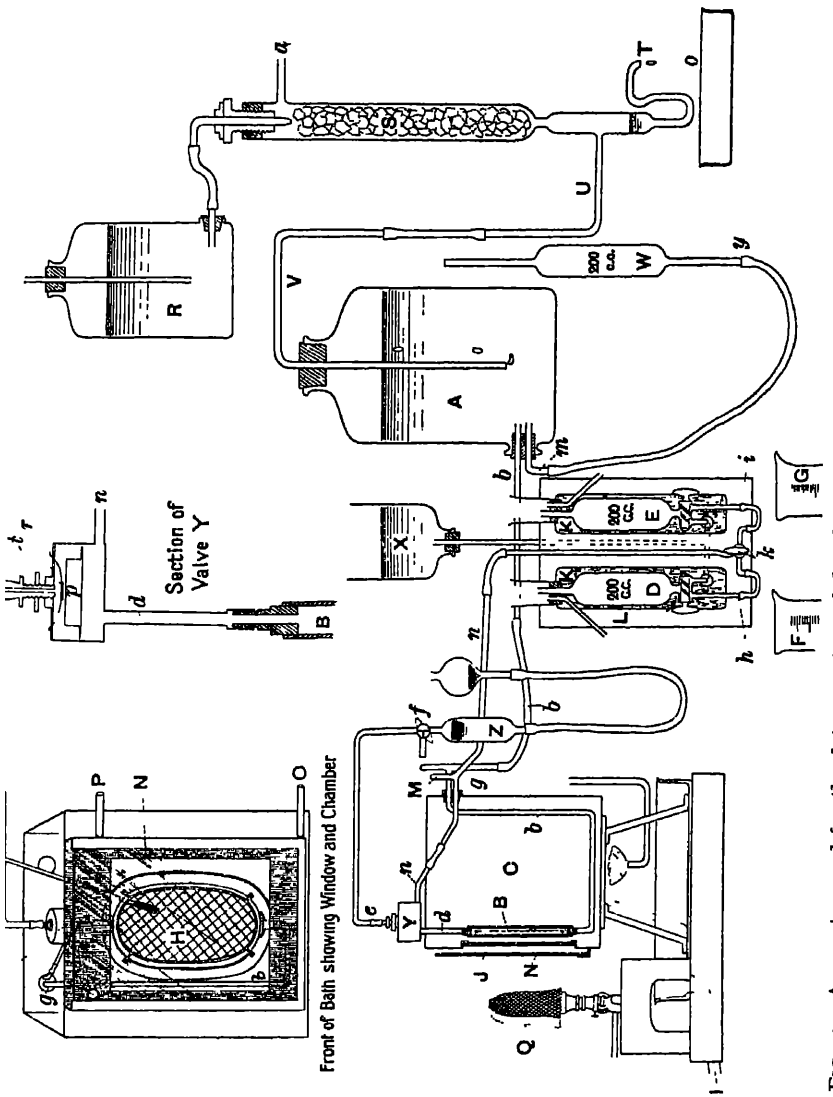

FIG 5.—Apparatus used for the determination of the photosynthesis of water plants (Blackman and Smith)
(Reproduced by permission of Dr Blackman and the Council of the Royal Society)

which the water plant is held in position by attachment to a silver grid The arrangement of parts is shown in Fig. 5 The plant material is contained in the chamber B provided with glass sides

The stream of water flows from the reservoir A through the tube *b* into the chamber B, which is contained in a bath of constant temperature and illuminated by light of desired intensity. After leaving the assimilation chamber by the tube *d*, the water stream passes through the tube *n* into one or other of the two 200 c.c pipettes D and E, which when full overflow into the measuring cylinders F and G. By means of the latter the velocity of the water stream is measured, and irregularities in the velocity can be prevented by raising or lowering the pipettes. The carbon dioxide content of the water was determined by addition of excess of standard baryta water and back titration with standard hydrochloric acid.

For applying the necessary correction for respiration similar experiments must be conducted in the dark, the conditions being otherwise the same as in the assimilation experiments The value found for the respiration must then be added to that found experimentally for the apparent assimilation, as already explained.

3. *Gas Analysis Method applied to Water Plants.*—A method applicable only to water plants has been used and described by Warburg and Negelein (1922), who worked with *Chlorella* A glass trough is two-thirds filled with a suspension of the alga, and then, after being filled with air containing carbon dioxide, is connected with one arm of a Barcroft differential manometer (Barcroft, 1908). To the other arm of the manometer is connected a trough containing only a salt solution of the same composition as that containing the algæ. Any pressure changes recorded by the manometer are then independent of temperature and atmospheric pressure. In order to determine the change in carbon dioxide and oxygen content of the troughs, the apparatus is shaken, the manometer read and the gas analysed. If oxygen has given place to an equal number of molecules of carbon dioxide the pressure decreases, since carbon dioxide is more soluble in water than oxygen, while the reverse is the case if oxygen replaces carbon dioxide If P is the total pressure in the vessel at the beginning of the experiment and P′ the total pressure at the end, T the absolute temperature and p the saturation pressure of water vapour at T°, if α_{O_2} and α_{CO_2} are the absorption coefficients of oxygen and carbon dioxide respectively in the suspension fluid at T°, v_F and v_G the volumes of the cell suspension and gas respectively, b_{O_2}, b_{CO_2} and b_{N_2} the percentage content of the gas mixture in oxygen, carbon dioxide and nitrogen before the experiment, and b'_{O_2}, b'_{CO_2} and b'_{N_2} the corresponding values after the experiment, then the quantities of oxygen and carbon dioxide *evolved*, x_{O_2} and x_{CO_2} respectively, are given by

$$x_{O_2}=\left[v_G \cdot \frac{273}{T}+v_F\alpha_{O_2}\right]\frac{P-p}{76{,}000}\left(\frac{b_{N_2}}{b'_{N_2}} \cdot b'_{O_2}-b_{O_2}\right)$$

and

$$x_{CO_2}=\left[v_G\cdot\frac{273}{T}+v_F a_{CO_2}\right]\frac{P-p}{76{,}000}\left(\frac{b_{N_2}}{b'_{N_2}}b'_{CO_2}-b_{CO_2}\right)$$

A negative value indicates absorption of the gas, a positive value evolution

4. *Change in Alkalinity Method*.—A simple method of measuring the photosynthesis of water plants, depending on the increase of alkalinity of a weak solution of a bicarbonate containing assimilating plant material (cf p. 47) has been described by Osterhout and Haas (1918*a*, *b*, also Osterhout, 1919). The method was used successfully with a marine alga, *Ulva*, and with fresh-water plants including *Spirogyra*, *Hydrodictyon* and *Potamogeton*.

In experiments with *Ulva* the alga was exposed to sunlight in sea-water contained in a closed glass tube, and the alkalinity produced determined by the addition of a definite quantity of phenol phthalein. Or the indicator can be added to the sea-water containing the alga before the commencement of the experiment, it having been found by control experiments that the indicator does not affect the amount of photosynthesis in the concentrations employed. The pink colour of the sea-water was then compared with that of a series of similar tubes containing the same concentration of indicator in a series of buffer solutions of known alkalinity. The amount of photosynthesis corresponding to different degrees of alkalinity was then determined by making simultaneous determinations of the oxygen evolved by means of Winkler's method, and of the alkalinity As a matter of fact, it was found that over the range of values involved, the amount of oxygen evolved is approximately a linear function of the change in P_H value of the sea-water, so that this change in P_H value can be taken as a measure of the apparent assimilation The respiration can be measured by performing a similar experiment in the dark, and so the necessary correction to the apparent assimilation can be applied in order to obtain the value of the true assimilation

With fresh-water plants the assimilating material is contained in a gallon bottle filled with water containing a little phenol phthalein To this a solution of sodium bicarbonate is added drop by drop until a pink colour is produced The procedure is otherwise the same as in the case of *Ulva*

The method has recently been adapted for determining photosynthetic activity of brackish-water organisms (Bruce, 1924).

Methods depending on the Evolution of Oxygen

5. *The Bubble-counting Method*.—As we have noticed in the last chapter, the bubble-counting method was introduced by Dutrochet in 1837, but it was Sachs (1864*b*) who developed it as a method for measuring the rate of photosynthesis. It was

subsequently improved by Kohl (1897), and used in a number of researches (*e.g.* those of Treboux, 1903, Pantanelli, 1903, and Kniep and Minder, 1909), but as Kohl left it the method contained numerous defects to which attention has been drawn more than once, and particularly by Kniep (1915), who subjected the method to a very thorough critical examination.

The principle of the bubbling method as a quantitative method consists in taking the number of bubbles evolved by an assimilating submerged water plant in unit time as a measure of the rate of photosynthesis The experimental procedure often employed is characterised by an equal simplicity. A piece of a water plant, such as a cut shoot of *Elodea* or a detached leaf of *Potamogeton*, is tied to a small glass rod used as a weight to keep the material submerged, and is placed in water containing carbon dioxide in solution. On illumination bubbles escape in a continuous stream from the cut end of the shoot or leaf-stalk, and can be collected in an inverted test-tube or other vessel if so desired

Carbon dioxide can be dissolved directly in the water, or it can be supplied in the form of bicarbonate, which, as we have seen (p 46), on account of hydrolysis yields a supply of carbonic acid or carbon dioxide

It will be observed that the rate of bubbling will only give a true measure of the rate of assimilation after the usual correction for respiration, provided that (1) the bubbles are all of the same size, (2) the bubbles always contain the same proportion of oxygen, and (3) the oxygen escapes from the plant in the form of bubbles at the same rate as it is formed

It has been found by experience that none of these conditions actually holds The size of the bubbles depends principally on the area of the cross-section of the air space of the cut stem or leaf-stalk As this will vary, not only from species to species, but from individual to individual and among leaves and stems of the same individual, comparison of rates of assimilation of different objects is practically impossible with the method in its simple form Moreover, the size of the bubbles evolved from the same cut stem may not remain constant Alterations in the size of the aperture of the cut stem may result from changes in temperature or from changes in osmotic pressure of the external liquid, or from internal changes which bring about a change in the degree of turgidity and size of the cells surrounding the aperture Alterations in surface tension of the external liquid will also affect bubble size When assimilation is rapid the bubbles evolved are smaller than those given off when assimilation is slow

With regard to the composition of the bubbles, analysis of the gas evolved shows that a quantity of nitrogen is always present This is partly nitrogen displaced from the air in the intercellular spaces as oxygen passes into these latter from the assimilating cells, while more nitrogen may pass into the bubble from the

water during the passage of the bubble through this, especially in the later stages of a long experiment when the intercellular spaces contain higher proportions of oxygen than in earlier stages of the same experiment. It is clear that the proportion of nitrogen in the bubbles is not likely to remain constant over an experiment of long duration. Kniep (1915) found that when bubbling is rapid the percentage of nitrogen in the bubbles is considerably lower, and that of oxygen considerably higher, than when bubbling is slow The results of analyses are shown in the following table.—

TABLE 7

COMPOSITION OF BUBBLES FROM ASSIMILATING SHOOTS OF CABOMBA CAROLINIANA (Data from Kniep)

Gas	Percentages of various gases in bubbles when evolved at rate of 1 bubble in 1·7 sec	1 bubble in 0·44 sec
Carbon dioxide	1·5	2·2
Oxygen	22·8	40·2
Nitrogen	77·2	59·8

When water supersaturated with carbon dioxide is employed, bubbling may occur in the dark (Kniep, 1915). This stream of bubbles has, of course, nothing to do with assimilation, and, if not recognised for what it is, may lead to false conclusions

It is also the case that oxygen may pass out from the plant by diffusion through the water and not in the form of bubbles Water in contact and in equilibrium with the atmosphere contains only 21 per cent of the quantity of oxygen it contains when in equilibrium with pure oxygen Consequently, if a water plant is illuminated in water which has been in contact with air, a significant proportion of the oxygen evolved in assimilation will be absorbed by the water and reach the outer air by diffusion through the water. If the water is kept perfectly still a steady gradient of oxygen concentration will finally be established between the surface of the assimilating cells and the surface of the water in contact with the atmosphere, and diffusion of oxygen will proceed slowly and steadily It is clear that while this steady state is being reached, the rate of bubbling will gradually increase until, when the steady state of diffusion is reached, the rate of bubbling may be expected to remain steady also It is also clear that stirring the water, or even slightly shaking it, will disturb the oxygen concentration gradient and so bring about a temporary increase in the rate of diffusion, and correspondingly depress the rate of bubbling

The method has been much improved by Wilmott (1921), who succeeded in eliminating some of the more serious sources of error by simple means Errors due to variation in the size of the bubbles are prevented by the use of a small glass nozzle or "bubbler" which fits over the cut end of the shoot and is provided at its

upper end with a fine glass capillary opening The bubbles escape by way of this opening, which of course remains constant in size. This simple device eliminates errors due to variations in the size of the aperture of the cut shoot or leaf, whether brought about by osmotic action or otherwise, and those due to variations in the internal gas pressure. It is also possible, by using the same bubbler with different plants, to compare their rates of assimilation, which cannot be done otherwise

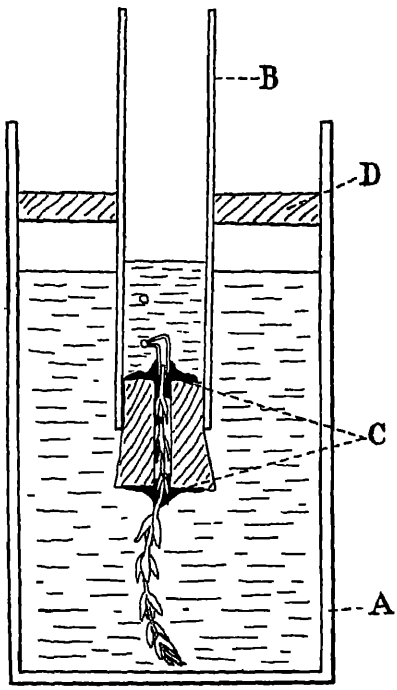

FIG. 6.—Wilmott's apparatus for the determination of photosynthesis by the bubble-counting method (Reproduced by permission of Dr F F Blackman, Mr A J Wilmott, and the Council of the Royal Society)

The complication arising from the effect of varying surface tension on bubble size is eliminated by another simple device The bubbles are not delivered into the experimental liquid, but into a cylindrical glass tube ("bubbling cup") containing distilled water, the surface tension of which, naturally, remains constant whatever the experimental solution The whole arrangement is shown in Fig 6 The tube B, 18 mm. wide in Wilmott's arrangement, is closed below by a cork C provided with a hole through which the inverted stem or leaf-stalk is pushed, and to which the latter is firmly attached by a wax mixture of low melting-point

The initial error introduced by the diffusion of oxygen through the water was eliminated by Wilmott by the use of water which had been charged with oxygen by prolonged violent shaking in bottles with an atmosphere of pure oxygen obtained from a cylinder Either standard strong carbon dioxide solutions were diluted with this water before use, or sodium bicarbonate was dissolved directly in it

6. *Blackman's Palladium Black Method*.—An ingenious method for measuring the oxygen evolved from land plants during assimilation has been devised by F F Blackman, but so far has only been described in outline (Briggs, 1920) The leaf is illuminated in a small closed chamber in an atmosphere of hydrogen and carbon dioxide After the experiment the gas in the chamber is circulated

over palladium black. The oxygen unites with twice its volume of hydrogen, and the reduction in volume of the gas is measured by means of a gas burette The reduction is three times the volume of oxygen produced, so that small amounts of oxygen can be measured The usual correction must be made for respiration

7. *Analysis by Means of Winkler's Method*—Where the photosynthesis of a submerged water plant containing no intercellular spaces is under examination and the oxygen evolved is so little that bubbles are not evolved, the determination of the oxygen produced may be made by Winkler's method for estimating dissolved oxygen. For the analytical procedure reference must be made to text-books of chemical analysis In using this method care must be taken that the experimental liquid does not come in contact with the external air, as if this happened there would at once be a tendency for the liquid and atmosphere to come into equilibrium, and oxygen would be absorbed by or given off from the liquid

If assimilation is only slight the experiment can be carried on in a vessel filled with water containing carbon dioxide in solution, and analysis of the liquid for oxygen at the end of the experiment is then sufficient to give a value of the apparent assimilation This arrangement is apparently that used by Harder (1921*a*) in experiments with *Fontinalis*, *Cladophora* and *Cinclidotus*, and in later experiments with *Phormidium foveolarum* in which Harder (1923*a*) expressly states that very small quantities of oxygen are involved.

An adaptation of Winkler's method, easy of manipulation and particularly suitable for work on photosynthesis, has been described by Osterhout and Haas (1917), in which the danger of contamination of the experimental liquid with external oxygen when removing the organisms or siphoning off a sample of the liquid, is avoided, and in which samples of the liquid can be taken for analysis from time to time.

Eudiometric Methods—The eudiometric methods employed to determine assimilation by measuring the change in carbon dioxide content of the gas in a closed vessel can be equally well employed for determining the output of oxygen. The method of Warburg and Negelein for determining the assimilation of submerged water plants similarly gives both the carbon dioxide absorbed and the oxygen evolved

Methods depending on the Formation of Carbohydrate

8 *The Dry Weight Method*—The method of measuring photosynthesis in which the gain in carbohydrate is determined is due to Sachs (1884), and is often described as the half-leaf method. At the beginning of an experiment one half of a leaf is cut from the rest so as to leave the other half attached to the midrib Pieces of the leaf of known area are then cut from the severed half, care

being taken to avoid the larger veins The dry weight of the pieces of leaf is then determined, so that a value for the dry weight of unit area of the leaf before the commencement of the experiment is obtained. The attached half-leaf is exposed to illumination under desired conditions, and at the end of a suitable time pieces of the leaf of known area are cut from this experimental half-leaf and the dry weight of these determined. The gain in dry weight is then attributed to the carbohydrates formed in assimilation Owing to translocation of the products of assimilation away from the leaf, data obtained in this way from half-leaves still attached to the tree need correction To obtain the true value for assimilation Sachs added the loss in dry weight per unit area during the night to the gain in dry weight per unit area found during the day. This should serve the double purpose of correcting both for respiration and translocation.

The dry weight method has been subjected to criticism by Brown and Escombe (1905*a*) and to a detailed review by Thoday (1909). Brown and Escombe, as noted earlier in this chapter, carried out determinations of photosynthesis by measuring the absorption of carbon dioxide from a continuous gas stream They found that Sachs obtained considerably higher values for photosynthesis by the dry-weight method than they obtained by direct determination of the carbon dioxide absorbed. To find a reason for this divergence they carried out determinations of photosynthesis of the same material by both methods In experiments with the leaves of *Catalpa bignonioides* they found by the dry-weight method an increase in dry weight, in the mean, of 6 69 mg per sq decimetre per hour, whereas the rate of formation of carbohydrate calculated from the intake of carbon dioxide on the assumption that 0·64 g of carbohydrate is formed from each gram of carbon dioxide absorbed, was, in the mean, only 2 35 mg per sq decimetre per hour. This very great divergence is attributed by Brown and Escombe to three sources of error in the dry-weight method These are: (1) possible changes in the power of the colloids to retain water on drying at 100° after assimilation; (2) differences in the venation and thickness of the two halves of the leaves, and (3) alteration in the area of the leaf as a result of insolation Should a leaf undergo shrinkage during illumination, the dry weight per unit area after illumination would be correspondingly increased, and too high values would be obtained for photosynthesis

With regard to the first of these possible sources of error, Thoday measured both the gain in dry weight and the gain in carbon content per unit area He found that the starch equivalent of the gain in carbon varied from 20 per cent less to 40 per cent more (neglecting one extreme case where the figure was 90 per cent. more) than the actual increase in dry weight of the same leaf Thoday concludes that the dry weight method is not vitiated by any large indeterminable errors arising through varying water-

retaining power of the leaf colloids after drying at 100° C But while Thoday's results show clearly that such an error will not account for the differences observed by Brown and Escombe, it seems possible that changes in the composition of the leaf during insolation might be responsible for a moderate error

With regard to errors arising from lack of symmetry of the two halves of a leaf, Brown and Escombe made a number of determinations of the dry weight per unit area of the two halves of the same leaf In *Catalpa bignonioides* the difference in the dry weight of unit area of the opposite sides of leaves was found to vary from 0·7 per cent to 5 7 per cent In other leaves, for example those of *Tropæolum majus*, smaller differences were observed, as, for example, 0 3 per cent in the species mentioned. Thoday obtained results similar to those of Brown and Escombe. He pointed out that the error due to lack of symmetry can be considerably lessened by using parts of leaves free from large veins, instead of using whole half-leaves He was thus able to reduce the difference in the dry weight of the two halves of the leaf of *Paulownia imperialis* from 5 95 per cent. (average of two pairs of measurements) to 1 4 per cent (average of four pairs of measurements).

The third source of error suggested by Brown and Escombe is also not negligible. They found the area of the leaves of *Catalpa bignonioides* might alter considerably during insolation, the changes varying from an increase of 0 14 per cent to a decrease of 3 12 per cent. Thoday found that leaves of *Helianthus annuus* might diminish in area by more than 5 per cent between early morning and midday under conditions favouring rapid transpiration

From the data provided by Brown and Escombe and Thoday it is clear than an error of 2 per cent in the determination of the dry weight of a half-leaf would be in no way extraordinary. In such a case Brown and Escombe show that with a leaf possessing a dry weight of 0 5 g per sq. decimetre and producing 0 002 g carbohydrate per sq decimetre per hour the error in the value of assimilation obtained in an experiment lasting 5 hours would amount to 100 per cent, whereas the error obtained by measuring the absorption of carbon dioxide would amount to no more than 2 per cent.

It is clear, therefore, that Sachs's dry-weight method is not very accurate But if it could be made so, it would prove a very valuable one, as the method is simple in principle and practice Thoday makes useful suggestions for increasing the accuracy of the method, but there is no doubt that it cannot compare with the gas-stream method for accuracy.

9 *Saccharification Method*—This method, recommended and used by Pollacci (1907*a*), has recently formed the subject of investigation by Miss Long (1919) Its essential characteristic is the determination of the reducing power by means of Fehling's solution of aqueous extracts of leaves or other assimilating tissue collected

at the beginning and end of an experimental period The difference between the reducing powers of the two extracts is taken as a measure of the amount of photosynthesis during the period

The plant material to be examined is either cut up in the fresh state or first dried and then powdered The finely divided material is then boiled in water to gelatinise starch, if present, and to extract the soluble matter After cooling, the mass is treated with taka-diastase to hydrolyse the starch The boiling and treatment with taka-diastase is repeated twice, and then, after a fourth boiling, the mass is treated with lead acetate, the excess of the latter removed with sodium carbonate, and the extract separated from the solid residue After boiling the extract with hydrochloric acid and neutralising with alkali until the extract is only slightly acid, the reducing power is determined gravimetrically by means of Fehling's solution, the assumption being made that the reducing power of the extract is entirely due to glucose

Miss Long made some determinations of photosynthetic activity by means of the method, and her results suggest that the method may be useful, but it is not clear at present that it possesses any very distinct advantage over the dry-weight method with its simpler technique.

CHAPTER VI

THE ENTRANCE OF CARBON DIOXIDE INTO THE ASSIMILATORY ORGANS

The Path of Gaseous Exchange

The entry of carbon dioxide into the green assimilating cells of any plant must take place by diffusion through the cell wall. The wall of living cells certainly contains a considerable quantity of water which quite probably forms the dispersion medium of a colloidal system, but however this may be, in all probability the carbon dioxide diffuses through this aqueous part of the cell wall.

In the algæ and the gametophytes of Bryophyta and Pteridophyta, we must suppose the entry of carbon dioxide into the assimilating organ takes place in the same way, as the whole of the surface of such organs is covered with cell wall, and the same must be the case in submerged aquatic plants generally. In the vast majority of the higher plants, including the sporophyte generation of the Pteridophyta, scattered over the surface of the assimilating organs are small openings, the stomata, which connect the outer atmosphere with the intercellular space system of the assimilating leaves or stems. It very naturally came to be regarded as likely that the path of entry of carbon dioxide into the leaf is through the stomata into the intercellular space system, from which the carbon dioxide diffuses in aqueous solution through the walls of the assimilating cells bordering the spaces. The oxygen evolved may be supposed to follow the same path, and the path of the gases absorbed and excreted in respiration will be the same Such an arrangement has two manifest advantages over diffusion through the surface cells of leaf and stem. In the first place, the carbon dioxide is brought into contact with the assimilating cells themselves and does not have to diffuse through the outer non-assimilating epidermal cells, and in the second place a very much greater absorbing surface is presented to it, the sinuous surface of the mesophyll cells intensifying this advantage On the other hand, the arrangement has the apparent disadvantage that the proportion of the actual surface

the stomata and intercellular spaces of the leaf by injecting these with water, so that carbon dioxide can only reach the assimilating cells by diffusion through water from the outer air. The result of this treatment on leaves of *Nerium Oleander*, in which the stomata are confined to the lower surface, is to reduce considerably the relative quantity of carbon dioxide diffusing through the stomatal surface Coating the under surface of the leaf with vaseline has a still more marked effect in this direction (cf Table II) These results thus confirm the conclusions derived from experiments with other methods.

TABLE II

EFFECT OF BLOCKING STOMATA BY WATER AND VASELINE ON THE RESPIRATION FROM THE TWO SURFACES OF LEAVES OF NERIUM OLEANDER

(Data from F F Blackman)

Condition of Leaf.	Respiration ratio, Upper surface / Lower surface
Normal	1·39
Injected with water	1·10
Under surface vaselined	1 3

ON THE RATE OF DIFFUSION THROUGH THE STOMATA

While the experiments described above leave no doubt that the path, or at any rate the main path, of gaseous exchange is through the stomata, there are facts which make it difficult to understand how this can be so For the concentration of carbon dioxide in the normal atmosphere is very low, namely, 3 parts in 10,000 (cf Brown and Escombe, 1905*b*, *c*), while, the fraction of leaf surface occupied by stomatal openings is also very small, and in spite of these facts the rate of assimilation of carbon dioxide by the leaf may be very considerable Thus, Brown and Escombe found that a leaf of *Catalpa bignonioides* can absorb from the atmosphere 0·07 c.c of carbon dioxide (measured at normal temperature and pressure) per sq cm of leaf surface per hour. The stomata in this leaf occupy 0 9 per cent of the whole leaf surface, so that diffusion of carbon dioxide through them must take place at the rate of 7·77 c.c. per sq cm per hour On the other hand, Brown and Escombe found that a normal solution of sodium hydroxide only absorbs from the same atmosphere under the same conditions about 0·120 c.c of carbon dioxide per sq cm of absorbing surface per hour, while if the air, instead of being moderately still, is made to move rapidly over the absorbing surface the rate of absorption is raised to a maximum of only 0 177 c c per sq cm. per hour If diffusion of carbon dioxide into the leaf takes place only through the stomata, the rate of absorption of carbon dioxide by the leaf must therefore be about 50 times as fast as the rate of absorption of the gas by normal sodium hydroxide solution. This appears at first sight impossible.

It was the difficulty presented by these facts that led Brown and Escombe (1900) to investigate the rate of diffusion through small apertures comparable with the stomata. They used for this purpose flat-bottomed flasks containing 200 c c. of normal sodium hydroxide with a surface about 10 cm in diameter, the mouth of the flask being closed except for an aperture of known dimensions This was achieved by passing the neck of the flask through the bottom of a small glass cup, to which it was cemented. The annular space between the neck of the flask and the side of the cup was filled with mercury and a flat-bottomed nickel crucible inverted over the mouth of the flask so that the edge of the crucible dipped into the mercury, thus securing a perfect seal The bottom of the nickel crucible was perforated by a hole of a known and desired size.

The results of Brown and Escombe's experiments are summarised in Table 12 From their results these workers concluded that with small apertures the rate of diffusion through the aperture is proportional, not to the area of the aperture, but to the diameter

TABLE 12

DIFFUSION OF CARBON DIOXIDE THROUGH APERTURES OF VARIOUS SIZES

(Data from Brown and Escombe)

Diameter of aperture in mm	CO_2 diffused per hour	CO_2 diffused per sq cm per hour	Relative areas of apertures.	Relative diameters of apertures.	Relative amounts of CO_2 diffused in unit time
22 7	0 2380	0 0588	1 00	1 00	1 00
12 06	0 0928	0 0812	0 28	0 53	0 39
12 06	0 1018	0 0891	0 28	0 53	0 42
6 03	0 06252	0 2186	0 07	0 26	0 26
5 86	0 05558	0 2074	0 066	0 25	0 23
3 233	0 03988	0 4855	0 023	0 14	0 16
3 216	0 03971	0 4852	0 020	0 14	0 16
2 117	0 02608	0 8253	0 008	0 093	0 10
2 00	0 02397	0 7629	0 007	0 088	0 10

This result may be understood by considering the lines of flow of the carbon dioxide towards an absorbing disk in the two cases where the disk is large and where it is small In the former case, assuming the air is still, the concentration of the carbon dioxide will increase from zero at the surface of the disk to its maximum concentration theoretically at an infinite distance from the surface, but which is practically reached at, say, 10 or 20 cm from the disk The disk being large, the carbon dioxide will diffuse equally rapidly towards the plane surface over the greater part of it The so-called " shells " of equal carbon dioxide density are plane surfaces parallel to the absorbing surface and the lines of flow of the gas will be at right angles to the surface Under these conditions we should expect the absorption to be proportional to the surface Only at the margins will a complication arise, as here carbon dioxide

will be drawn in from the air at the side of the disk, and we should therefore expect in the region of the margin that the absorption of carbon dioxide per unit area would be higher than in the middle of the disk, as a larger volume of gas is drawn upon by unit area of the surface in this region But if the surface is a large one, this "marginal absorption" will be only a negligible fraction of the whole, and we should expect, therefore, in the case of a large absorbing surface, that the absorption would, to all intents, be proportional to the area. In the case of a small disk, on the other hand, the "marginal absorption" will form a greater proportion of the whole

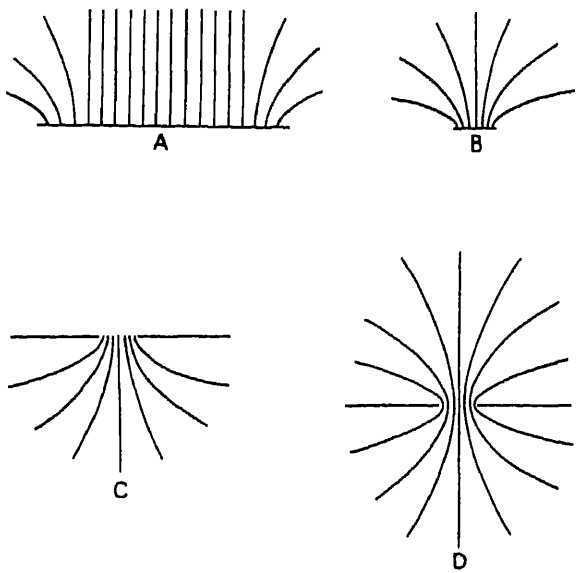

FIG. 7.—Diagrams indicating the lines of flow of a gas towards an absorbing surface or through an orifice A, lines of flow towards a large plane surface, B, towards a small absorbing surface, both in still air, C, lines of flow inside a small orifice when the concentration of the gas outside the orifice is kept constant by a rapid current of air, D, lines of flow of a gas outside and inside a perforation in perfectly still air

the smaller the disk, so that with very small surfaces we may expect the absorption by the disk to be proportional to the marginal region, which is, of course, proportional to the linear dimensions of the aperture and not to the area Exactly similar considerations hold for the diffusion of carbon dioxide or any other gas through an aperture.

The diagrams in Fig 7 may help to make these considerations clear. In A are shown the lines of flow of a gas in the case of a large absorbing surface Here, except in the marginal region, which is negligible in comparison with the whole area, the flow of

gas is at right angles to the surface, and the absorption will clearly be proportional to the area. In B are shown the lines of flow in the case of a small absorbing surface. Here the region over which the lines of flow are at right angles to the surface is negligible in comparison with the marginal region outside the area over the middle of the disk, and consequently the absorption will be proportional to the length of the margin, which will itself, in the case of a circular opening, be proportional to the diameter. In C are shown the lines of flow through a small aperture opening into a large space where the gas is absorbed, the concentration of the gas being kept at a constant value outside the opening by means of a strong current of air. In D is represented the case corresponding to diffusion through a stoma, diffusion being supposed to proceed from still air outside the perforation into a still space inside Actually there is nearly always some movement of air outside the stomata under natural conditions, so that the actual state of affairs in regard to a stoma will be intermediate between the cases represented by C and D

Particular attention has been paid to the problems of evaporation of water from surfaces of different sizes and under different conditions, and it will be obvious that the problems are really the exact converse of the problems of absorption by a surface Stefan (1882) found that the evaporation of water from a small circular disk into still air is given by the formula

$$Q=4ka \log \frac{P-p''}{P-p'}$$

where Q is the quantity of liquid evaporated in a certain time, k is the coefficient of diffusion of the water vapour, P is the pressure of the atmosphere, and p' and p'' are the pressures of the vapour at the surface and at an infinite distance from it respectively, and a the radius of the surface The rate of evaporation is thus proportional to the diameter, and the same law will hold for the converse to evaporation, the absorption of a gas at the surface The formula thus agrees with results obtained experimentally by Brown and Escombe and those from *a priori* considerations as to the proportionality between absorption of the gas and the linear dimensions of the absorbing surface

In the case of diffusion through a stoma we are dealing with a perforation having a depth which is not negligible in comparison with the diameter For such a case Brown and Escombe deduce the rate of diffusion through a perforation, the air being still throughout the system, as given by the formula $\frac{k\rho\pi a^2}{l+\frac{1}{2}\pi a}$, where k is the diffusivity of the gas, ρ the density of the gas in the air just outside the pore, l the depth of the pore and a its radius.

The laws of evaporation from, and hence also of absorption by,

a surface when the air above is in movement, have been investigated mathematically by Jeffreys (1918) This worker found that, within certain limits, when a steady wind is blowing over a flat surface of water the rate of evaporation from areas of the same shape will be approximately proportional to $a^{1.5}$, where a is proportional to the linear dimensions of the surface For a circular area of radius a the rate of evaporation is $3\,95\rho V_0(kua^3)^{\frac{1}{2}}$, where ρ is the density of the air, V_0 the partial pressure of the vapour at the surface, k the effective conductivity of the vapour, u the velocity of the air, and a the radius of the surface In the outer air, with a wind blowing over the surface at about 400 cm per second, the limits of surface between which this relation would hold are in the neighbourhood of 10 cm. and 250 metres radius In a room where there is only little draught and the air moves at about 4 cm. per second, the limits are about 1 cm. and 25 metres When, however, $\frac{ua}{k}$ is much less than unity, as will be the case with a stoma, the rate of evaporation becomes proportional to the linear dimensions as in still air

The result that for surfaces of medium dimensions the rate of evaporation is proportional to the square root of the cube of the radius of the surface in moving air agrees with the result obtained experimentally by Thomas and Ferguson (1917*a*, *b*), and agrees on the whole with the results of Renner (1910) on evaporation [1]

Jeffreys further found that the rate of evaporation from a circular cylinder wet at the bottom and open at the top is equal to $\frac{\pi a^2 k\rho V_0}{l+\frac{1}{4}\pi a}$, where l is the depth of the cylinder and a its radius, and the other symbols have the significance previously assigned to them Thus when the depth of the cylinder is great compared with the radius, the rate of evaporation will be approximately proportional to the area of the evaporating surface, but when the radius is large in comparison with the depth, the rate of evaporation will be more nearly proportional to the radius (or diameter) In moving air the rate of evaporation is

$$\frac{\rho V_0}{\frac{l}{a^2k}+\frac{1}{3\,95(kua^3)^{\frac{1}{2}}}}$$

These results agree with those found experimentally by Brown and Escombe and by Thomas and Ferguson respectively It is to be expected that absorption of carbon dioxide will obey the same laws Thus both in still and in moving air the rate of diffusion through a stoma depends on the length of the stomatal pore as well as on its diameter

[1] The conclusion of Sierp and K L Noack (1921) that evaporation in wind is proportional to the area of the surface is not justified by their experimental data (Stiles, 1924*a*)

It has to be emphasised that the diffusion through any one stoma cannot be considered as independent of the other stomata on the surface of a leaf. This becomes clear both from the experiments of Brown and Escombe and from the mathematical investigation of Jeffreys referred to above Brown and Escombe investigated the diffusion of carbon dioxide through multiperforate septa consisting of sheets of celluloid 0 08 to 0·1 mm thick, through which holes of a required diameter were punched at definite distances from one another The septa were fixed to the open end of glass tubes containing sodium hydroxide, and the rate of diffusion of carbon dioxide through these multiperforate septa measured as in the observations on the rate of diffusion through single small pores. The results obtained are summarised in Table 13.

TABLE 13

DIFFUSION OF CARBON DIOXIDE THROUGH SEPTA PERFORATED WITH PORES 0 380 MM IN DIAMETER, OPENING INTO A TUBE OF LENGTH 1 0 CM AND CONTAINING A SOLUTION OF SODIUM HYDROXIDE AT THE BOTTOM

Distance of pores apart in diameters.	Number of pores per sq cm. of septum	Percentage of area of septum occupied by pores.	Diffusion through septum stated in percentage of diffusion through the open tube
2 63	100 00	11 34	56 1
5 26	25 00	2 82	51 7
7 8	11 11	1 25	40 6
10 52	6 25	0 70	31 4
13 1	4 00	0 45	20 9
15 7	2 77	0 31	14 0

From a comparison of the two columns on the right it will be observed that the reduction in the rate of diffusion resulting from the presence of the septum is very considerably less than the reduction in the area through which diffusion can take place Thus when the pores only occupy 1 25 per cent of the area of the septum, the diffusion is as much as 40 6 per cent of that which takes place through the open tube; that is, the diffusion is about 32 times as much as it would be if it were proportional to the area Such a result is naturally to be expected from the diameter law for diffusion through small apertures already dealt with.

From their results Brown and Escombe conclude that as the distance between the pores increases the efficiency of each pore for diffusion increases, until the distance between the holes is about 10 times the diameter of a pore As will be seen from Table 13, the diffusion is then about 45 times as great as it would be if it were proportional to the area of the pores, and it will be observed that with increase in the relative distance between the pores the relative efficiency does not increase further Brown and Escombe therefore conclude that when the pores are 10 diameters or more apart the mutual interference of the density shells of carbon dioxide above the pores is negligible, and each pore acts practically independently

of the neighbouring ones according to the diameter law When the pores are nearer together than this there is interference, the carbon dioxide above one pore being within range of what would be the lines of flow of the gas through a neighbouring pore, if the latter were alone acting.

In some cases, at any rate, the stomata of a leaf are about this distance apart from one another. In the leaf of *Helianthus annuus*, for example, they are stated to be 8 diameters apart The under surface of such a leaf thus forms a multiperforate septum in which the pores are so far apart that each pore can exercise almost its full efficiency with regard to the diffusion of carbon dioxide through it Brown and Escombe work out the capacity of the leaf for absorbing carbon dioxide on this assumption, taking the average diameter of a stoma as 0 00107 cm , the length of the tube within the stoma as 0 0014 cm , the number of stomata per sq cm as 33,000, and the area of cross-section of a stoma as $9\ 08 \times 10^{-7}$ sq cm They calculate that the leaf in rapidly moving air could absorb 2·578 c c of carbon dioxide per sq cm. of leaf surface per hour, while in still air this value would be reduced to 2 095 c c per sq cm per hour. As these values are far higher than any observed rate of carbon dioxide absorption by the leaf, these results, as far as they go, indicate that the stomata present more than sufficient area to allow all the carbon dioxide absorbed by the leaf to pass into the latter through them in spite of their small size and in spite of the low concentration of the gas in the atmosphere.

Although this conclusion is not affected by Jeffreys' more recent mathematical treatment of the problem, yet this latter writer calls in question some of Brown and Escombe's conclusions. Thus Jeffreys thinks that Brown and Escombe were in error in concluding that " the interference of the density shells of small holes set at 10 diameters or more apart is small, each hole beyond this limit acting almost independently according to the diameter law " On the contrary, Jeffreys concludes that if there are more than 600 stomata per sq. cm the rate of evaporation (and hence also of absorption) " must be enormously restricted by the presence of other stomata." In general, in still air, Jeffreys concludes that only so long as n^2al is less than unity, where n^2 is the number of the stomata per sq. cm , a the radius of the stoma, and l is of the order of the linear dimensions of the leaf, will each stoma act independently of the others This means that, with a leaf about 3 cm long and with 33,000 stomata per sq cm , diffusion through a single stoma only becomes independent of that from the others when the radius of the stomatal aperture is less than 10^{-5}. Now, as the diameter of a stoma on the leaf of *Helianthus annuus* is, as we have seen, about 10^{-3}, Jeffreys concludes that the stomata have to close until the diameter is only $\frac{1}{60}$ of the full aperture before they act independently of the neighbouring ones, and he thinks this explains Lloyd's conclusion (1908) that the regulatory function

of stomata is almost nil This opinion is so contrary to the generally accepted view of stomatal functions that experimental verification of it seems necessary before accepting it

When the quantity n^2al is greater than unity the rate of absorption will be the same as if the whole leaf is an absorbing surface, and consequently the diameter law will not hold In such a case the total diffusion into the leaf is not a function of the number of stomata, but the diffusion through any one stoma is inversely proportional to the total number This fact was recognised by Renner (1910, 1911*a*, *b*), who states that evaporation from a leaf is the same as that from a water surface of the same dimensions.

In moving air, Jeffreys comes to the conclusion that in this case also, with stomata open at full aperture and with the usual conditions of their distribution over the surface, the total evaporation from, and hence also absorption by, the stomata will not be very different from the case in which the whole surface of the leaf is evaporating or absorbing, as the case may be. The total evaporation or absorption will then be proportional to $l^{1.5}$, where l is proportional to the linear dimensions of the leaf, as this latter will in general fall within, or not much outside of, the limits within which this law holds When the stomatal aperture or number of stomata per unit area is much smaller, or when the rate of movement of the air is very considerable, the conditions may approach more nearly those in which the diameter law is operative and each stoma acts independently of the others so that the number of stomata determines the total diffusion through the leaf surface.

As the evaporation from, or absorption by, the stomata cannot be greater than that which would take place if the whole surface of the leaf were active, Jeffreys concludes that the best way of determining the possible maximum rate of evaporation or absorption in any particular case is to calculate the rate, both on the basis of the sum of the evaporations or absorptions from individual stomata, and on the basis of the whole leaf as the evaporating or absorbing surface The smaller of the two results obtained will give the maximum evaporation or absorption of which the leaf is capable

It is clear from these theoretical considerations that the stomata constitute a very efficient path for the diffusion of carbon dioxide into the leaf, and that they are capable of allowing carbon dioxide to pass through them at rates many times greater than those that have been actually observed.

In conclusion, it should be pointed out that a reconsideration of the problem of the passage of carbon dioxide into the leaf appears to be wanted. The systems discussed by Brown and Escombe and later writers are rather generalised systems differing in structure somewhat from those of the leaf Further, it has to be remembered that in many cases diffusion through the cuticle may be active, so that the path of absorption of carbon dioxide is not limited to the stomata.

CHAPTER VII

THE INFLUENCE OF EXTERNAL AND INTERNAL CONDITIONS ON PHOTOSYNTHESIS

General Remarks

In the course of development of our knowledge of photosynthesis it was discovered, as described in Chapter I, that various conditions are necessary for photosynthesis to take place The most obvious of these conditions are a supply of carbon dioxide in the external medium, light, a suitable temperature and chlorophyll. As water is necessary for the production of carbohydrate when carbon is provided in the form of carbon dioxide, a supply of water is a further condition Also, as merely exposing carbon dioxide and water to light in presence of chlorophyll does not give rise to carbohydrate, it appears that a further condition of photosynthesis is bound up with the living protoplasm What this condition is cannot be said with any definiteness It may be some particular substance present in the protoplasm, or it may be related to the structure of the living substance , at present we have to be content with describing it as the protoplasmic factor or component in photosynthesis (Blackman, 1923) Nor is it possible to say whether this so-called protoplasmic factor is resolvable ultimately into more than one.

The conditions mentioned above are the only conditions or factors known definitely to be essential for photosynthesis It is, however, possible that there are others Thus it has been thought that a small quantity of oxygen is necessary for the commencement of photosynthesis. If the presence of oxygen is indeed a factor, it is only necessary to supply it at the commencement of an experiment, as, once photosynthesis has commenced, oxygen is provided by the process itself. Also a supply of certain salts may possibly be necessary Whether this is so or not, it appears that the supply of nutrient salts may affect the magnitude of the photosynthetic process, while there is evidence that particular substances, such as acids and ammonium salts, may also influence the rate of photosynthesis. There is, of course, a possibility that other substances may affect photosynthesis

The conditions which influence, or possibly influence, the rate of photosynthesis may very conveniently be divided into two

groups, external and internal Apart, however, from those conditions which are known to be essential to the process, there are others which call for consideration, either because they influence photosynthesis or because they are possibly essential. The factors of photosynthesis to be discussed are as follows :—

Essential external factors.

1. Carbon dioxide concentration.
2 Light intensity.
3 Temperature.
4 Water supply.

Other external factors

5 Wave length of incident light.
6 Supply of nutrient salts
7 Osmotic pressure of the medium (in the case of water-plants).
8 Oxygen.
9 Various other substances.
10 Wounding.
11 Electrical conditions

Internal factors

12 Chlorophyll content
13 The protoplasmic factor.
14 Anatomical structure
15. The accumulation of the products of assimilation

In the course of this chapter our knowledge of the action of these various factors will be reviewed, the factors being considered in the order indicated above.

The Interaction of the Factors

As a result of earlier investigations on the relation between the various external conditions and photosynthesis, it came to be generally accepted that there is a *minimum* value of the condition below which photosynthesis does not proceed With increase in the condition photosynthesis proceeds more rapidly until a value of the condition, the *optimum,* is reached, at which photosynthesis proceeds most rapidly With further increase in the value of the condition the rate of photosynthesis becomes less and less, until a value of the condition, the *maximum,* is reached, above which photosynthesis does not take place at all. This general conclusion is well expressed by Pfeffer (1900) when he says, for example, " The curve of assimilation rises as the temperature increases, and remains fairly constant at an optimum approximating to that for growth. Above this optimum the assimilatory curve falls again " Similar statements are made with regard to the influence of light and carbon dioxide supply.

The reasons for the decline in the rate of photosynthesis in values of any condition above the optimum were not always clear

In the case of carbon dioxide supply it was realised that high concentrations of the gas exert a poisonous effect on the plant (cf. Grischow, 1819, Boussingault, 1868; Pfeffer, 1900), while in the case of light, Pfeffer supposed that prolonged exposure to too intense illumination brings about an inactivation of the chloroplasts Prolonged exposure to temperatures above the optimum are supposed to produce the same effect It can, however, be urged with a considerable degree of truth that there is little explanation in these statements

Obviously one would expect the minimum, optimum and maximum points to be different for different species These cardinal points in the case of temperature, for instance, one would expect to be lowest in arctic plants and highest in those of the tropics, while the range of values between the minimum and maximum may also be expected to vary But apart from these expected variations, this conception of minimal, optimal and maximal values of each condition was soon found to present difficulties. This can be illustrated by a remark of Pfeffer with regard to the influence of carbon dioxide on photosynthesis, to the effect that " there is a certain optimal percentage at which assimilation is most active, and this varies not only in different plants, but also according to the external conditions, for both the intensity of the illumination and the rapidity of gaseous exchange must influence the assimilatory curve Thus when the stomata are closed, assimilation may be active only when the percentage of carbon dioxide present in the external air is abnormally high, whereas under normal conditions a slight increase in the percentage of this gas is sufficient to produce the maximal activity of carbon dioxide assimilation."

Thus Pfeffer realised that the optimum value was not a fixed value in the case of any condition with any one plant species, but might depend on other conditions This view of a variable optimum depending on the values of other factors operative was further developed by Pantanelli (1903) as a result of work done in Pfeffer's laboratory With such an outlook on the relation between the various conditions of photosynthesis, the term "optimum" appears to lose much of its significance and definiteness. Indeed, Blackman and Smith (1911*b*), in criticising Pantanelli's work, refer to this outlook as a transitional view between the old clear-cut view of the definite optima, and the view put forward by Blackman in which the term "optimum" is dispensed with altogether

To F. F. Blackman is due the complete realisation of the necessity for taking into account all the other factors when the influence of any one particular factor on photosynthesis is examined An example of the manner in which Blackman conceived the factors to interact may be quoted in illustration. A green leaf is exposed to such illumination as will provide energy to decompose exactly 5 c.c. of carbon dioxide an hour If the concentration of carbon

dioxide outside the leaf is such that only 1 c c of carbon dioxide is assimilated in an hour, the energy provided by the light is sufficient for the whole of this carbon dioxide to be utilised in photosynthesis. If the concentration of carbon dioxide is now raised so that 2 c c. of this gas can be utilised, the energy is still more than sufficient to allow the utilisation of the full quantity of carbon dioxide In both these cases the carbon dioxide is limiting the rate of photosynthesis, while energy is present in excess. Now suppose the concentration of the carbon dioxide is raised so that 5 c c of the gas can diffuse into the leaf in an hour The energy supplied is now just sufficient to allow the assimilation of the carbon dioxide If the concentration of carbon dioxide is further increased no further increase in the rate of assimilation can take place because the energy supply is now insufficient, as the energy supplied is only capable of decomposing 5 c c of carbon dioxide in an hour. The

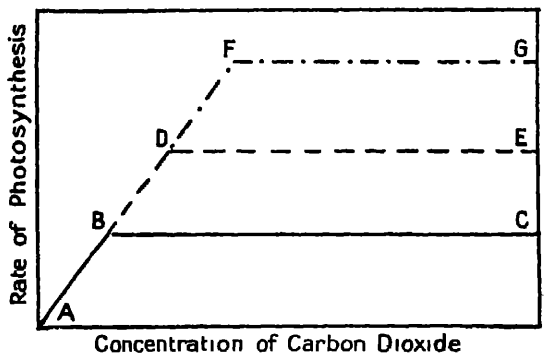

FIG. 8 —Scheme to illustrate the action of a limiting factor. (After F F Blackman)

light intensity is now limiting the rate of photosynthesis, while the carbon dioxide is in excess The relation between carbon dioxide concentration and light intensity is shown graphically by the curve ABC in Fig. 8 Along the part AB the rate of assimilation is limited by the concentration of carbon dioxide As the latter increases the rate of assimilation increases, and if the rate of assimilation is directly proportional to the carbon dioxide concentration the part of the curve AB will be a straight line At the point B the light is just sufficient to enable the full utilisation of carbon dioxide, while along the part BC of the curve increase in carbon dioxide supply brings about no further increase in the rate of assimilation because the supply of light energy is insufficient to enable more carbon dioxide to be assimilated than that corresponding to the point B The curve connecting assimilation and carbon dioxide supply is thus composed of two parts, a rising part

in which the assimilation is limited by the carbon dioxide supply, and a part parallel to the horizontal axis corresponding to the limitation of assimilation by the light intensity. In the first part of the curve light is supplied in excess, in the latter part of the curve carbon dioxide At the point B, where a break in the curve occurs, neither is in excess. Now, if the light intensity be increased to double its former value a correspondingly greater quantity of carbon dioxide can be utilised, and consequently, as the concentration of carbon dioxide is increased above that corresponding to the point B, the assimilation will also increase until the light again imposes a limit to the rate of assimilation, and the curve connecting carbon dioxide concentration and rate of assimilation will now be ADE. Similarly, with a still stronger light intensity the curve would be AFG.

These considerations make it clear that the influence of carbon dioxide concentration on rate of assimilation cannot be investigated without regard to light intensity, and obviously other conditions must equally be taken into account In particular, care must be taken that no factor other than the one under consideration is limiting the rate of assimilation In the example given above, for example, the part BC of the curve ABC is determined, not by the carbon dioxide concentration, the factor under consideration, but by the light intensity.

Blackman (1905) was thus led to enunciate the "principle of limiting factors" as follows: "When a process is conditioned as to its rapidity by a number of separate factors, the rate of the process is limited by the pace of the 'slowest' factor." In the case quoted above, carbon dioxide concentration is the limiting factor, the slowest factor, over the ascending part of the curve, and light intensity the limiting factor over the horizontal part The work of Blackman and his pupils was first directed to determining the relations of the essential external conditions, carbon dioxide concentration, light intensity and temperature, to photosynthesis The magnitude of the latter, according to Blackman, is determined in every combination of these factors, by one of them acting as a limiting factor To discover which is the limiting factor the following principle can be applied. "When the magnitude of a function is limited by one of a set of possible factors, increase of that factor, and of that one alone, will be found to bring about an increase of the magnitude of the function."

Blackman and Smith (1911*b*) have constructed curves indicating the relation between photosynthesis and these three external factors when each one is limiting in the case of *Elodea* These are indicated in Fig 9. A line drawn horizontally through the figure cuts the three curves in points which correspond to the lowest value of each of the three factors necessary to give the rate of photosynthesis indicated by the point where the line cuts the vertical axis.

The principle of limiting factors should be of general application to all processes which depend on a number of conditions It is, indeed, similar to Liebig's "Law of the Minimum" applied to crop yield by Liebig eighty years ago, and even to the "Law of Population" enunciated by Malthus in 1798 (cf also Jorgensen and Stiles, 1919).

Nevertheless, during recent years there has been a considerable expression of dissent from the principle of limiting factors Thus H D Hooker (1917), in a discussion of the principle of limiting factors and the law of the minimum, comes to the conclusion that

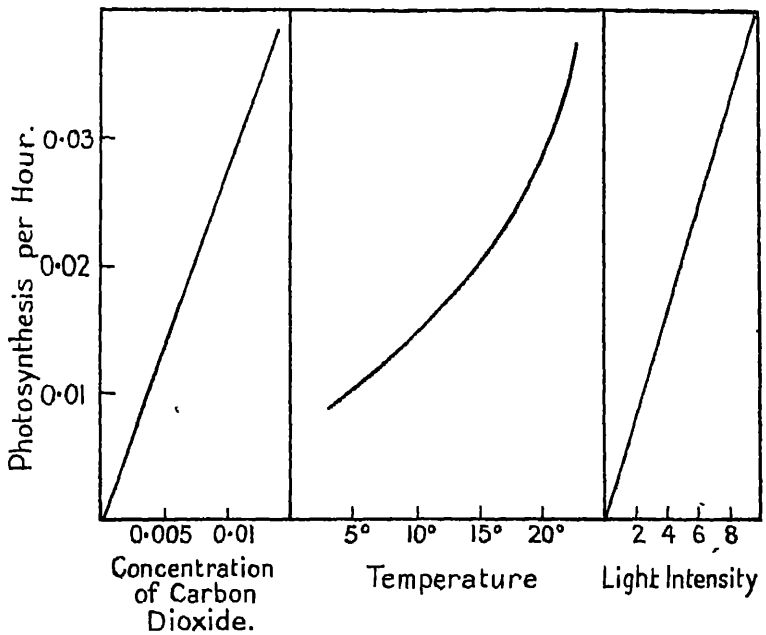

FIG 9 —Inter-relation of environmental factors and rate of photosynthesis in *Elodea* (After Blackman and Smith)

only individual processes obey the law, which does not apply to questions of general development In commenting on this paper Crocker (1918) urges that the principle of limiting factors should not be applied too rigidly to physiological processes In particular, the action of stimuli "renders the conception of limiting factors less definite," while "the conception of an external condition as a limiting factor frequently leads physiologists to fail to examine the internal mechanism upon which that and other factors play to bring about a given result"

These criticisms are against the application of the principle of

limiting factors to plant activities in general A criticism of the principle applied to the particular case of photosynthesis was made in 1918 by W H. Brown The chief point in this writer's criticism depends on the interpretation of the results of experiments made by Blackman and Smith on the assimilation of *Elodea* to which reference has already been made Blackman and Smith carried out experiments with varying concentrations of carbon dioxide with two light intensities (5 7 and 8 1 units) The temperature was generally about 19° C. Blackman and Smith interpreted their results as indicating a relationship between carbon dioxide supply and photosynthesis similar to that indicated by the curve

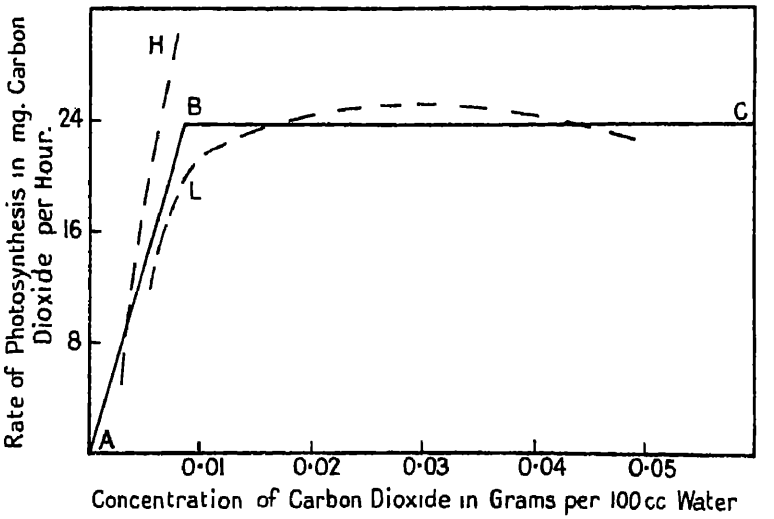

FIG 10 —The relation between concentration of carbon dioxide and rate of photosynthesis according to the data of Blackman and Smith The interpretation of these workers is shown by the continuous line, that of W H Brown by the broken lines (After Blackman and Smith and W H Brown)

ABC in Fig 8 The actual curve they give is shown by the continuous line in Fig 10 Over the ascending part AB of the curve carbon dioxide supply is the limiting factor, so that with increase in carbon dioxide concentration there is an increase in the rate of photosynthesis, but with further increase in carbon dioxide concentration beyond that corresponding to the point B, there is no further increase in photosynthesis because light is now the limiting factor In criticising this interpretation of the experimental results, Brown constructs two separate curves from the experimental data provided by Blackman and Smith, one curve to represent the experiments carried out with 5 7 units of light, and

the other to represent those with 8 1 units. The curves drawn by Brown in this way are shown by the broken lines in Fig 10, that for the lower light intensity being denoted by L and that for the higher light intensity by H Neither curve shows the operation of a limiting factor A similar criticism of other work by Matthael (1904), supposed to show the action of limiting factors, is made by Brown and Heise (1917*a*), who hold that the experimental results in this case also are wrongly interpreted

The whole point at issue is really the determination of the curve which represents correctly the relation between a particular factor and the rate of assimilation Owing to variability of the assimilating tissue used for the different experiments, the actual values obtained do not lie exactly on the curve proposed by Blackman and Smith, nor on those constructed by Brown. Perhaps the most correct statement that can be made in the light of Brown's criticism of the principle of limiting factors is that the results of Blackman and his pupils agree approximately with the principle, but, owing to the variability of the plant material used, the data are insufficient to decide whether the principle is obeyed rigidly and exactly or whether the relation between any particular factor and photosynthesis is rather like that suggested by Brown It should be pointed out that Blackman himself realised the difficulty, and he says, " Physico-chemical finality is not to be attained in this matter, but special research might at least show how far the recorded optima for assimilation and respiration are real metabolic truths and how far they are illusions of experimentation " Also Smith (1919), in a counter-criticism of Brown, says, " It is not claimed that a limiting factor curve always adheres rigidly to a typical form with a sharp angle at the point of change of the limiting factor. It is conceivable, and is indeed probable, that when, so to speak, two factors are close to the limiting value, a change in the one not limiting may have some appreciable effect on assimilation. This will show itself about the inflexion of the curve where the limiting factor is changing " This statement would presumably cover the interpretations of their results by the continental writers whose work is discussed below

The continental writers referred to have carried out measurements of photosynthesis and have been unable to obtain any indication of a break in the curve indicating the relation between a factor and the rate of photosynthesis As an introduction to this further consideration of the principle of limiting factors the work of Boysen Jensen (1918) may be cited with advantage Boysen Jensen observed the assimilation of *Sinapis alba, Senecio sylvaticus, Rumex Acetosella* and *Sambucus nigra* in different intensities of light In each case he expresses the results graphically in the form of a smooth curve (cf. Fig 11) which follows a course described by Boysen Jensen as follows: " The curve begins below the axis of abscissæ, which is crossed at some distance from the

zero point, the first part of the curves is about linear, next they bend gradually and become finally about parallel to the axis" There is no indication of the rigid operation of the principle of limiting factors, for there is no sharp break in the curve at the point where light ceases to be the limiting factor and one of the other essential factors limits photosynthesis But, as Boysen Jensen realised, the variability in the material is rather great, and it would be possible to regard the actual values as lying on a curve with a sharp break like those in Fig 8 Boysen Jensen himself says: "On the whole the curves are identical with those found by Blackman for the influence of light on the CO_2 assimila-

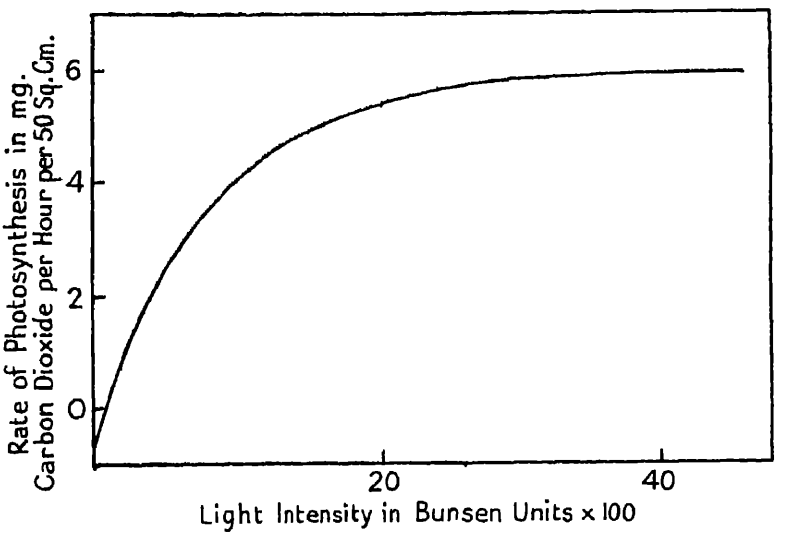

FIG 11.—Curve to illustrate the relation between light intensity and rate of photosynthesis in *Sinapis alba*. (After Boysen Jensen) Correction has to be made for respiration

tion. In the first part light is the limiting factor, and in the last part another factor (temperature or CO_2 supply) is limiting. Between these two parts there is a third part of considerable extent where the CO_2 assimilation is neither constant nor proportional to the light intensity, here the different factors interact." Boysen Jensen's view is thus not identical with that of the principle of limiting factors in its original form, for in the latter there is only one point at which both of two factors under consideration can be regarded as limiting, this is at the point at which the break occurs On Boysen Jensen's view the factor which is relatively more in the minimum chiefly determines the rate of assimilation, but as it increases relatively to another factor the latter begins to exert an

effect as well until when this is in relative minimum the assimilation is practically proportional to it.

This view is practically a modification of the principle of limiting factors to some extent similar to the modification of Liebig's law of the minimum propounded by Mitscherlich (1909, 1911, 1919, 1921) in the following terms: "The amount of the crop rises on increasing the supply of a nutritive substance acting as limiting factor, but the increase is not proportional to the supply, but decreases gradually when approaching the point where the factor in question is no more limiting" (Boysen Jensen, 1918)

The same view with regard to the interaction of factors in photosynthesis has been put forward more recently by Benecke (1921, 1924) and Harder (1921*a*) Benecke adopts Mitscherlich's terminology and refers to a factor being in "absolute minimum" when it is zero With low values of the factor it is in "relative minimum," and with increasing value of the factor photosynthesis follows an approximately logarithmic course until the factor is in "relative optimum," by which Benecke means that value of the factor which gives the highest rate of photosynthesis when all other factors are maintained constant The magnitude of this "relative optimum" is conditioned by the mutual relation of all the other factors, so it is no fixed value

On the rigid definition of the principle of limiting factors it has already been mentioned that increase in the value of the limiting factor, and of that alone, will produce an increase in assimilation Benecke cites an experiment in which *Elodea* was assimilating at such a rate that 10 bubbles of gas were evolved in 90 seconds when exposed to light at a distance of 40 cm from a lamp and immersed in 0 1 per cent potassium bicarbonate When the light intensity was increased by reducing the distance from the lamp to 30 cm , the rate of assimilation was increased so that now 10 bubbles were evolved in 30 seconds, while on reducing the light intensity by increasing the distance of the assimilating plant from the lamp to 50 cm bubbling ceased The carbon dioxide supply was now increased by replacing the potassium bicarbonate solution by one ten times as concentrated, and bubbling then recommenced at the rate of 10 bubbles in 120 seconds. It would therefore appear that both increase in carbon dioxide supply and in light intensity would bring about an increase in the rate of assimilation, so that two factors were limiting in the way indicated by Boysen Jensen It must, however, be pointed out that Benecke's observations are not numerous, while the method he used is one that, without the improvements introduced by Wilmott (cf Chapter V), is not likely to yield trustworthy results, as, indeed, the actual numbers given by Benecke would suggest

A stronger case in support of Boysen Jensen's modification of the principle of limiting factors has been made out by Harder (1921*a*) Harder used chiefly the aquatic moss *Fontinalis anti-*

number of observations were made on leaves of *Glyceria spectabilis*, *Typha latifolia* and *Nerium Oleander*, and one series of experiments with each of *Prunus laurocerasus* and *Myagrum perfoliatus* Godlewski concluded that with increase in carbon dioxide content of the air surrounding the leaf, the rate of assimilation increased up to a certain limit When the concentration of carbon dioxide is increased beyond this optimum value the rate of assimilation is less The rate of falling off of assimilation with increase in carbon dioxide concentration above the optimum is, however, much slower than the rate of increase in the process with increasing carbon dioxide concentration below the optimum The optimum value varies with the species. For *Glyceria spectabilis* it was found to lie (for clear days between May 28 and July 1 at Wurzburg) between 8 and 10 per cent., for *Typha latifolia* between 5 and 7 per cent , and for *Nerium Oleander* probably somewhat lower

Similar results were obtained by Schutzenberger and Quinquaud (1873) in experiments with water-plants

Kreusler (1885), using an electric lamp as source of constant illumination, and a current of air containing carbon dioxide as source of the latter, found in experiments with leaves of *Rubus*, *Carpinus* and *Tropæolum*, that with increasing concentration of carbon dioxide the rate of photosynthesis increased up to a point, but with further increase in carbon dioxide concentration photosynthesis fell off. The optimum carbon dioxide concentration appeared to be in the neighbourhood of 10 per cent.

None of these workers took any factors other than carbon dioxide supply into consideration, and in view of the discussion in the previous section of this chapter it will be clear that no definite value can be placed on these determinations of optima Nor are the values actually obtained numerous enough to enable any decision to be made with regard to the actual relation between carbon dioxide concentration and rate of photosynthesis

The work of later authors indicates that, at any rate in low concentrations of carbon dioxide, that is, where carbon dioxide is the limiting factor or factor in relative minimum, the rate of photosynthesis is directly proportional to the carbon dioxide concentration. Thus, Brown and Escombe (1902) found that in the leaves of *Helianthus annuus*, photosynthesis is directly proportional to the concentration of carbon dioxide up to a concentration of 8 mg per litre, that is, fifteen times the concentration of the gas in the atmosphere In water-plants Treboux (1903) and Pantanelli (1903) obtained similar results with the bubble-counting method. Blackman and Smith (1911*b*), using as source of carbon dioxide a continuous stream of water charged with the gas, found that with *Elodea* and *Fontinalis* assimilation increased in proportion to the carbon dioxide concentration until light became a limiting factor, when the assimilation remained constant at a value determined by the light intensity Lundegårdh (1921) also

found that in concentrations of carbon dioxide up to 1·71 mg. per litre the rate of photosynthesis of leaves of a number of different species exposed to light of varying intensities ($\frac{1}{40}$ to $\frac{1}{4}$ full sunlight) was approximately proportional to the carbon dioxide concentration Differences were, however, found between the behaviour of sun and shade leaves, the limit of carbon dioxide concentration above which further increase of photosynthesis does not take place often depending probably, according to Lundegårdh, on closure of stomata as well as on light intensity Some values actually found by Lundegårdh are shown in the following table :—

TABLE 14

PHOTOSYNTHESIS IN $\frac{1}{40}$ SUNLIGHT AT 18° C. BY LEAVES OF *Oxalis Acetosella* IN DIFFERENT CONCENTRATIONS OF CARBON DIOXIDE

(Data from Lundegårdh)

Concentration of carbon dioxide in mg. per litre	Carbon dioxide assimilated in mg per 50 sq cm per hour	Carbon dioxide assimilated / Carbon dioxide concentration
0·57	0 45	0·79
1 14	0 9	0 79
1 71	1 35	0 79

Warburg (1919), working with *Chlorella*, found that the photosynthesis is proportional to the carbon dioxide concentration within the limits of 0 05 and 10, where unit concentration is taken as that of carbon dioxide in water in equilibrium with the atmosphere Above this upper limit further increases in carbon dioxide concentration result in smaller and smaller increases in photosynthesis, until the latter remains constant with further increase in carbon dioxide concentration Warburg concludes with Blackman that some other factor is now limiting, but as he used a favourably high temperature, namely 25° C , and a good light intensity, that of a 300-watt lamp at a distance of 20 cms , he considers it is an internal factor which is the limiting one, and this internal factor he hypothesises as a substance which reacts with the carbon dioxide.

While most workers are agreed that in lower concentrations of carbon dioxide the rate of photosynthesis in objects as different as *Chlorella* and foliage leaves is approximately proportional to the carbon dioxide concentration, different opinions are expressed with regard to the influence of higher concentrations on photosynthesis This matter has already been discussed in the preceding section of this chapter, where it has been pointed out that Blackman and Smith consider that assimilation increases proportionately to the carbon dioxide concentration until some other factor becomes limiting, while Harder concludes that the rate of increase in assimilation velocity continually decreases with increasing carbon dioxide, so that the curve connecting photosynthesis with carbon dioxide concentration is a smooth one of logarithmic type without

any break Warburg's results with *Chlorella* are rather similar, but the curve rises much more steeply at first, so that, as already stated, assimilation is roughly proportional to carbon dioxide concentration, the curve becoming flatter and approximately parallel to the axis of carbon dioxide concentration sooner than in the case of Harder's curves Some of Lundegårdh's results also suggest this falling off in the ratio between rate of photosynthesis and carbon dioxide concentration, but the differences between these results and those which would be obtained if assimilation were proportional to the carbon dioxide concentration may be within the limits of experimental error

A point worthy of notice in the results of Blackman and Smith is that when carbon dioxide concentration is the limiting factor, the rate at which *Fontinalis* assimilates is about half that at which *Elodea* absorbs carbon dioxide, while the results obtained by the same workers with *Ceratophyllum* and *Potamogeton* are of the same order as those obtained with *Elodea* Blackman and Smith suggest that the difference between the assimilatory activity of these phanerogams and that of the moss *Fontinalis* may be due to there being less obstacle to the diffusion of carbon dioxide up to the chloroplasts in the former plants However this may be, it is important to remember that the photosynthesis of carbon dioxide by a green organ is not a single process It is obvious that at least two processes are concerned (as we shall see later, there are certainly three), namely, the diffusion of carbon dioxide from the external medium to the chloroplast, and a process at the chloroplast (or other absorbing surface) in which the carbon dioxide is absorbed. Warburg, indeed, selected the unicellular alga *Chlorella* as the object of his investigations, because in such a minute object the concentration of carbon dioxide at the surface of the chloroplast must be in approximate equilibrium with that in the water outside, so that complications introduced by the diffusion stage are practically eliminated Warburg appeared to think that in the experiments of Blackman and Smith the rate of photosynthesis might have been determined by the rate of diffusion This, however, does not appear likely The rate of diffusion of carbon dioxide at constant temperature will be determined by the diffusion gradient, provided any obstacles in the path of diffusion remain the same The diffusion gradient will be determined by the concentrations of carbon dioxide in the external medium and at the absorbing surface Now if the rate of absorption is so rapid that the concentration of carbon dioxide at the absorbing surface can be taken as zero, we may regard the rate of diffusion as approximately proportional to the concentration of carbon dioxide in the external medium In this extreme case, therefore, the rate of diffusion would increase proportionately as the concentration of carbon dioxide in the external medium increased, and it would be impossible for diffusion velocity to account for the constancy of

assimilation with increase in carbon dioxide concentration in the external medium A retardation in the rate of diffusion of carbon dioxide into the leaf may, however, result as a secondary effect of high concentrations of carbon dioxide, as will be noted below.

The " optimum " recorded by earlier workers requires explanation. Blackman and Smith found in their experiments with water plants that no depression of photosynthesis occurs even with such a high concentration of carbon dioxide as 0 0536 per cent , which is an environment containing as much carbon dioxide as an atmosphere containing 30 per cent of the gas With higher concentrations, however, photosynthesis is less with increasing carbo dioxide concentration Blackman considers this to be a narcot effect of strong carbon dioxide : an effect which has been noted b a number of workers (cf. Chapin, 1902). This depression o assimilation is, in consequence, to be regarded as a secondary effect. A further reason for depressed assimilation in land plants in high concentrations of carbon dioxide is to be found in the action of carbon dioxide on the stomata. It has been observed (Linsbauer, 1916 , Chapman, Cook and Thompson, 1924) that carbon dioxide in high concentrations induces a closure of the stomata. If Jeffreys' conclusions (cf. p 72) are accepted, it would appear that a very considerable closure of the stomata must take place before the rate of diffusion of carbon dioxide into the leaf is reduced on account of this Nevertheless, it may be effective in reducing the rate of photosynthesis in high concentrations of carbon dioxide

Thus the whole curve expressing the relation between the rate of photosynthesis and the concentration of carbon dioxide when other external factors are kept constant may be regarded as consisting of three parts The first ascending part of the curve corresponding to the state of affairs where, with increasing carbon dioxide concentration, photosynthesis increases proportionately, is a straight line, or approximately one This passes, with or without a break, into a part parallel, or approximately so, to the axis of carbon dioxide concentration and corresponds to conditions where some factor other than carbon dioxide concentration is limiting (or in relative minimum) This region passes over into the third and descending part of the curve, where, with increasing carbon-dioxide supply, photosynthesis is lessened on account of narcotic poisoning, and possibly by closure of the stomata in high concentrations of the gas The level of the curve in the middle region will depend on the value of a factor or factors other than carbon dioxide concentration

Light Intensity

While a considerable amount of work has been done on the influence of light intensity on photosynthesis, many of the results

obtained are of doubtful value on account of faulty methods of experimentation. In earlier work, also, no correction was made for respiration To such earlier work it will, therefore, be necessary to make no more than passing reference

The first systematic researches on the influence of light intensity on photosynthesis appear to be those of von Wolkoff (1866) who exposed plants at various distances from an illuminated sheet of ground glass, and determined the rate of photosynthesis by means of the bubble-counting method. From his work, which involved the use of only low intensities of light over a small range, he concluded that photosynthesis is directly proportional to the light intensity. The results of a single experiment made by van Tieghem (1869), also by the bubbling method, are usually regarded as supporting the same conclusion On account of faulty method of experimentation the work of N. J C. Muller (1872, 1876) and of Famintzin (1880) need not detain us The work of the latter author was adversely criticised by Reinke (1883*b*), who, by means of the bubble-counting method, made a lengthy investigation of the influence of light intensity on the photosynthesis of *Elodea* A wide range of light intensities was employed, these varying from $\frac{1}{16}$ of to 60 times full sunlight The higher intensities were obtained by concentrating sunlight by means of a lens Reinke concluded that in moderate light intensities his results agreed with those of von Wolkoff in indicating an approximate proportionality between light intensity and rate of photosynthesis; with stronger intensities, however, there was a progressively smaller increase in the rate of photosynthesis for each successive unit increase in light intensity, this becoming noticeable at an intensity of about full sunlight With intensities of twice full sunlight and higher the rate of assimilation remained practically constant We may suppose that here some other factor was limiting, or in relative minimum In still higher light intensities a falling off in the rate of bubble emission was observed Some experiments made by Timiriazeff (1889) on the photosynthesis of *Potamogeton lucens* and some land plants have been criticised by Pantanelli (1903), also on account of the experimental arrangement, but the results, obtained by the eudiometric method, are very similar to those of Reinke They indicate again an approximate proportionality between photosynthesis and light intensity in low intensities of illumination (up to about one-fifth of direct sunlight), and then with further increase in light intensity the increase in the rate of photosynthesis becomes progressively less and less until in a light intensity of about one-half that of direct sunlight it remains constant with further increase in intensity of illumination

Kreusler (1885) also found the rate of photosynthesis approximately proportional to the light intensity.

The work of Pantanelli (1903) on this subject was also with

a water plant (*Elodea*), and the bubble-counting method was employed. His results were very similar to those of Reinke and Timiriazeff, in that in low light intensities (again up to about one-fifth of direct sunlight) the rate of assimilation was approximately proportional to the light intensity Above this limit further increase in the intensity of illumination produced very little increase in the rate of photosynthesis Pantanelli's results thus agree in essentials with those of earlier workers in indicating an approximate proportionality between photosynthesis and light intensity in low intensities of illumination, while in higher light intensities the rate of photosynthesis is determined by some other factor which is limiting

Pantanelli's results are, like most of those made earlier, open to the criticism that they were obtained by the unreliable bubbling method They have also been criticised on the ground that the temperature was not kept constant (Blackman and Smith, 1911*b*), so that this may have been a disturbing factor and have introduced an error. Brown and Heise (1917*b*) minimise this source of error on the ground that the influence of temperature is so slight that any error introduced by failure to keep the temperature constant may be disregarded, but, as will be shown in the next section of this chapter, the evidence points to temperature having a considerable influence on the rate of photosynthesis

The influence of light intensity on the rate of photosynthesis of a sun plant, *Œnothera*, and a shade plant, *Polypodium*, was examined by Weis (1903) The determinations of the assimilation were made by the eudiometric method of Bonnier and Mangin, while light intensity was measured by means of photographic paper As the rays of light which affect photographic paper are not those chiefly absorbed by chlorophyll, and so are unlikely to be effective in photosynthesis, this method of measuring light intensity is not ideal, although it may be adequate The results obtained by Weis are summarised in Table 15

TABLE 15

INFLUENCE OF LIGHT INTENSITY ON PHOTOSYNTHESIS OF SUN AND SHADE PLANTS

(Data from Weis)

Light Intensity (Full sunlight = 1)	Rate of Photosynthesis	
	Œnothera.	Polypodium
1 0	0 1660	0 0650
0 017	0 0517	0 0705
0 011	0 0270	0 0420

These results also indicate that in lower light intensities photosynthesis is proportional to the light intensity In full sunlight, in both plants, undoubtedly some other factor, very possibly carbon dioxide concentration, is limiting the rate of photosynthesis.

The difference between the results obtained with sun and shade plants will be discussed at a later stage.

An extensive series of experiments on assimilation of leaves of *Helianthus tuberosus* under natural conditions of illumination was made by Blackman and Matthaei (1905) The details of a typical experiment may be quoted The continuous gas current method was used The gas passing through the leaf chamber contained 2·5 per cent. of carbon dioxide, and passed through the chamber at the rate of 800 c c per hour The temperature was 18 0° to 18 3° C and the date of the experiment July 30, 1904 The results are shown in the accompanying table

TABLE 16

RATE OF PHOTOSYNTHESIS OF A LEAF OF *Helianthus tuberosus* EXPOSED TO NATURAL ILLUMINATION IN JULY

(Data from Blackman and Matthaei)

Time p.m.	Conditions of illumination	Temperature of bath in Centigrade degrees	Real assimilation per sq decimetre of leaf surface per hour in grams
12 30–1 30	—	—	Preliminary
1 30–2 30	Heavy clouds	18·2	0 0030
2 30–3 30	Violent thunderstorm at first, then clearing up slowly	18 3	0·0060
3 30–4 30	Brighter; no rain	18 3	0 0118
4 30–5 30	Sun at first, then clouded over, storm driving up	18·3	0 0086
5 30–6 30	Overcast, steady rain, heavy storm at 6 10	18 0	0·0020

It will be observed that the photosynthesis during each of the hourly periods varied markedly in the same direction as the light intensity as indicated by the atmospheric conditions. It is to be noted that in full sunlight under the conditions of the experiment the rate of assimilation of the leaf of *Helianthus tuberosus* is 0 0186 grm of carbon dioxide per sq decimetre of leaf surface per hour, so that in all the measurements recorded in Table 16 light intensity was a limiting factor.

From these experiments it was concluded that, provided temperature is sufficiently high, and carbon dioxide supply is in excess, the rate of photosynthesis varies with the intensity of illumination. For every temperature there should be a minimum light intensity which is sufficient to permit the maximum rate of photosynthesis at that temperature, presuming that neither carbon dioxide concentration nor any other factor is limiting By using perforated screens in front of the leaf to cut off a definite fraction of the sunlight, Blackman and Matthaei determined what fraction of full sunlight was required for the maximum rate of photosynthesis to take place. They found that at a temperature of 29 5° C.

during the middle hours of an August day in Cambridge, the maximum assimilation was obtainable by 0 36 of full sunlight with cherry laurel leaves (*Prunus laurocerasus*), while with *Helianthus tuberosus* the minimum light intensity necessary to give the maximum rate of photosynthesis was 0 69 of full sunlight Blackman and Mathaei found that when light intensity is the limiting factor equal areas of the leaves of different species exposed to the same intensity of illumination assimilate the same amount of carbon dioxide. The same law was found by Blackman and Smith (1911*b*) with aquatic phanerogams It is therefore to be expected that *Helianthus* is capable of a much higher rate of assimilation than cherry laurel at the same temperature This, as we shall see in the next section of this chapter, was found to be the case

A few experiments made by Blackman and Smith (1911*b*) on *Elodea* supplied with the carbon dioxide of a constant stream of water containing a known concentration of the substance, indicate that photosynthesis is proportional to light intensity when this is a limiting factor

The work of Boysen Jensen (1918) on this subject has already been mentioned in an earlier section of this chapter. As there pointed out, Boysen Jensen interprets his results as indicating that the relationship between photosynthesis and light intensity can be expressed as a smooth curve, there being no break between the first part of the curve corresponding to low light intensities where photosynthesis is approximately proportional to light intensity, and the part of the curve where in higher light intensities the influence of some other factor is felt Similar curves were obtained by Warburg (1919) with *Chlorella*, but his curves approach more nearly those of Blackman, for though, indeed, there is no break in the curve, the intermediate portion between the initial part in which photosynthesis increases proportionately to light intensity and the latter part in which, with increasing light intensity, photosynthesis remains practically constant, is shorter.

Lundegårdh (1921), in an investigation having ecological interests as primary consideration, obtained a somewhat different relation between light intensity and photosynthesis in woodland shade plants and in shore sun plants. Thus, with varying light intensity and constant carbon dioxide concentration of 0·57 mg. per litre, that of the gas in the normal atmosphere, he found in shade plants (*Oxalis Acetosella, Melandrium rubrum, Circæa alpina*) photosynthesis increased proportionately to the light intensity up to a light intensity of 0·05 to 0 1 of direct sunlight In light intensities exceeding this limit further increase in the value of this factor brought about no change in the rate of photosynthesis. The curve connecting light intensity and photosynthesis in these cases is thus of the typical Blackman form. If, however, the concentration of carbon dioxide was raised above that of the normal atmosphere, an increase in the rate of photosynthesis was observed

even in light intensities below 0·1 of direct sunlight, in a light intensity of 0 025, for example

In sun plants, *Nasturtium palustre* and *Atriplex latifolium*, the relation between light intensity and rate of photosynthesis was found to be different In low light intensities photosynthesis was again found to be approximately proportional to the intensity of illumination, but with increasing light intensity the curve between rate of photosynthesis and intensity of illumination was found to follow a more logarithmic course The difference in behaviour of sun and shade leaves is attributed by Lundegårdh to differences in anatomical structure of the two kinds of leaves, a question which will be discussed later in this chapter

Experiments by Stålfelt on photosynthesis in sun and shade leaves of fruit trees (1920) and of *Pinus sylvestris* and *Picea excelsa* (1921), gave, on the whole, similar results, but even in the shade leaves of these Coniferæ the maximum rate of photosynthesis was only attained in full daylight

Lundegårdh sees in this difference between the photosynthetic relations of sun and shade leaves an ecological significance. Shade plants live in a habitat in which the carbon dioxide content is about double that of normal air, so that, in spite of the lower light intensity, these plants are in a position to photosynthesise relatively large quantities of carbon dioxide, for even in low light intensities an increase in the concentration of carbon dioxide above that of normal air brings about an increase in the rate of photosynthesis From the purely plant physiological point of view his results agree fairly well with those of Boysen Jensen Lundegårdh's eudiometric method, although convenient, is perhaps not above criticism (cf Harder, 1921*b*).

Some measurements of the rate of photosynthesis in some ferns made by Johansson (1923) gave results which are interesting in comparison with those of Lundegårdh The experiments were carried out in air of normal carbon dioxide concentration The relation between rate of photosynthesis and light intensity in *Polypodium vulgare* was found to be that typical of a shade leaf, while *Pteris aquilina* behaved like a typical sun plant in this connection. In *Dryopteris austriaca* the relation between photosynthetic velocity and light intensity was found to be similar to that in *Polypodium vulgare* at low light intensities, but at a light intensity of 30 per cent. full daylight the rate of photosynthesis declined with further increase in intensity of illumination, so that in full daylight the rate of photosynthesis was found to be only 6 per cent of that at 30 per cent daylight A similar relation was found to hold in the case of *Dryopteris spinulosa*, maximum photosynthesis being observed in this fern at 60 per cent. full daylight. These results may possibly be attributable to the influence of light on stomatal aperture.

Experiments with *Fontinalis* were performed by Harder (1921*a*),

in which the carbon dioxide supply and temperature were kept constant and the light intensity varied As source of carbon dioxide tap water without addition of bicarbonate was used, and the temperature only varied between 23 0° and 24 0° C. Assimilation was determined by estimation of oxygen by Winkler's method. His results are summarised in Table 17 and are shown graphically in Fig 12 Like those obtained by all other investigators, these results indicate that in low light intensities photosynthesis is almost proportional to the light intensity. In higher intensities, however, there is a progressively smaller increase in the rate of photosynthesis for each unit increase in light intensity, so that the curve indicating the relation between photosynthesis and light intensity is smooth without any break.

TABLE 17

INFLUENCE OF LIGHT INTENSITY ON THE RATE OF PHOTOSYNTHESIS OF *Fontinalis*

(Data from Harder)

Light intensity in metre-candles	Real Assimilation in arbitrary units.
667	1 156
2,000	3 30
6,000	7·00
18,000	9 88
36,000	11·74

The relation between carbon assimilation and light intensity is thus very similar to the relation between carbon assimilation and carbon dioxide concentration In low light intensities there is fairly complete unanimity of opinion that photosynthesis is approximately proportional to the light intensity, while in high light intensities some other factor limits, or largely determines, the value of assimilation There is the same difference of opinion as in the case of carbon dioxide concentration as to whether the first part of the curve passes over gradually into the last part, or whether there is a sharp break in the curve where light intensity ceases to be the limiting factor

There is very little reference in the literature of the subject to a falling off of assimilatory activity in very intense light, similar to that which occurs in strong concentrations of carbon dioxide Such a falling off of photosynthesis was observed by Reinke in *Elodea* only when the intensity of illumination surpassed sixty times sunlight Pantanelli, however, working with the same species, found a falling off in assimilation when the light intensity was greater than that of full sunlight, and he considered the curve representing the relation between photosynthesis and light intensity to show an optimum which depended on the carbon dioxide content.

That Reinke did not obtain any retardation in the rate of

photosynthesis until such high light intensities were reached is thought by Ewart (1900) to be due possibly to the continuous rise of temperature of the water containing the *Elodea* plants used in Reinke's experiments. The increase in temperature would cause the expansion of the gases in the intercellular spaces, so that a stream of bubbles would result which had nothing to do with photosynthesis Ewart (1898*b*) has shown that living chloroplasts of *Elodea* and *Chara* lose their chlorophyll when exposed in cold water for 5 or 10 minutes to a light intensity 8 to 10 times that of

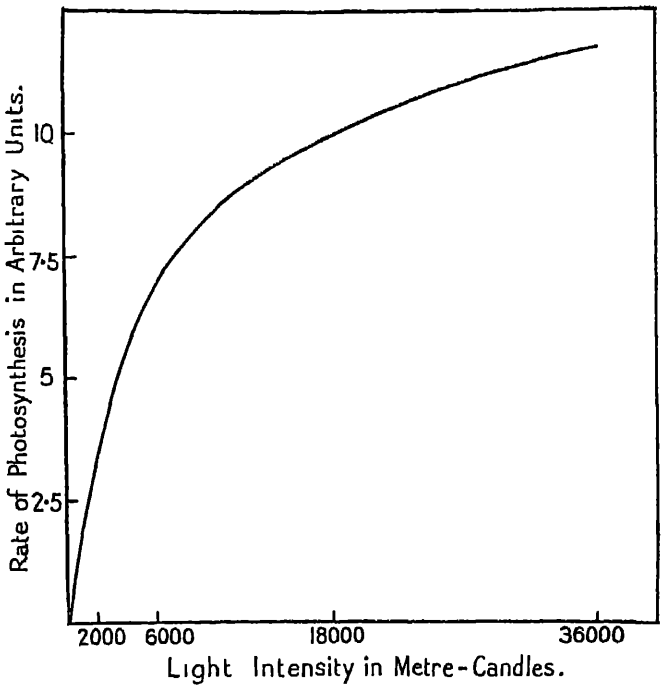

FIG 12 —Curve to illustrate the relation between light intensity and rate of photosynthesis in Fontinalis (Constructed from the data of Harder)

sunlight, so that in such a light intensity photosynthesis almost immediately stops on account of the injurious effect of the high light intensity on the chlorophyll. Leaves of many plants are much more resistant to prolonged exposure to direct sunlight, but many shade plants are comparatively sensitive and bleach on prolonged exposure to direct sunlight. On return to their normal surroundings the green colour returns

McLean (1920) and Yap (1920), working in the Philippine Islands with sugar-cane in the field, found that the rate of photo-

synthesis during the day increased rapidly from 7 30 a m to about 9 30 a m. and then fell somewhat to a minimum during the midday hours, rising to a maximum again in the neighbourhood of 5 p m. The midday depression occurred during a period of bright sunshine and at a time when the sun is most directly overhead, so it was suggested by McLean that the depressed rate of photosynthesis might be the result of excessive insolation On the other hand, it was recognised that something connected with the internal metabolism of the leaf might be responsible for the observed result

Solarisation —Ursprung (1917) found that leaves of *Phaseolus multiflorus* exposed to light do not form starch continuously, but after a time cease doing so, while dissolution of starch may even take place This effect is called "solarisation" by Ursprung The more intense the light the sooner it appears. After exposure to sunlight for 5 hours leaves contained abundant starch, but after insolation for 9 hours little starch was left Ursprung concludes that leaves are not adapted to too long periods of insolation, on the other hand, solarisation appears to have no permanently injurious effect on the activities of the leaf

The Compensation Point —It is interesting to note that as respiration is independent of light intensity, while assimilation increases with increase in light intensity, there will be a light intensity for every plant and every temperature in which the assimilation is exactly equal to the respiration, and in which, consequently, there will be neither evolution nor absorption of either carbon dioxide or oxygen This light intensity is called the *compensation point*

A number of values of the compensation point for different species have been determined by Plaetzer (1917), while some values are also given by Boysen Jensen (1918) These values are collected in Table 18

TABLE 18

COMPENSATION POINTS AT ABOUT 20° C

(Data from Plaetzer and Boysen Jensen)

Species	Compensation point	Observer.
Sinapis alba	1 0 Bunsen units × 100	Boysen Jensen
Senecio sylvaticus (sun plants)	0 6 " "	"
Rumex Acetosella	0 5 " "	"
Sambucus nigra (sun leaves)	0 7 " "	"
" " (shade leaves)	0 3 " "	"
Oxalis Acetosella	0 2 " "	"
Ajuga reptans	0 1 " "	"
Myriophyllum spicatum	128 lux [1]	Plaetzer
Cabomba caroliniana	55 "	"
Elodea canadensis (in summer)	2 "	"
" " (in winter)	18 "	"
Spirogyra sp.	174 "	"
Cladophora sp	253 "	"
Fontinalis antipyretica	150 "	"
Cinclidotus aquaticus	400 "	"

[1] See footnote, p. 129.

The values given in this table show what a very considerable variation exists among different species in regard to the value of the compensation point The reason for this wide variation is not at all clear Plaetzer showed that it is certainly not the case in water plants that the value of the light intensity at the compensation point is a function of the respiratory activity It seems clear from Boysen Jensen's results that the compensation point lies at a considerably lower light intensity in shade plants than in sun plants, while the same result was obtained by Lundegårdh (1921) Now Boysen Jensen found that the rate of respiration was considerably less in the shade plants examined (from 0 1 to 0 2 mg. carbon dioxide per hour per 50 sq. cm) than in the sun plants (from 0 3 to 0 8 mg. carbon dioxide per hour per 50 sq cm) noted in the table However, among the sun leaves alone and the shade leaves alone the compensation point is no more a function of the magnitude of the respiration than in the water plants examined by Plaetzer Lubimenko (1905, 1908) also examined photosynthesis in sun and shade plants He found that a sun tree, *Robinia*, required a much higher light intensity (25 times) for assimilation to exceed respiration than the shade tree *Fagus*, while similarly among conifers the sun tree the larch required ten times the light intensity for assimilation to exceed respiration than the shade tree the yew. Lubimenko thought this difference traceable to the higher chlorophyll content of the chloroplasts of shade plants This, however, is only a conjecture, and Lubimenko's experiments have been criticised by Benecke (1924) on account of the high concentration of carbon dioxide (8 per cent) used, but it is not certain that the gas would have a narcotic action in this concentration.

The Influence of Intermittent Illumination —This question has been investigated by Warburg (1919) in the case of *Chlorella* Intermittence of illumination was obtained by the use of rotating sectors, and the amount of photosynthesis during equal periods of illumination, not of equal experimental times, was compared It was found that in high light intensities the rate of photosynthesis in intermittent illumination is greater than in continuous illumination of the same intensity, the more rapid the alternation of light and dark periods the higher the rate of photosynthesis Thus with an alternation of 8000 per minute the rate of photosynthesis was found to be 100 per cent. above that in continuous illumination, while with an alternation of four light and dark periods per minute the rate of photosynthesis was only 10 per cent above that in continuous illumination. In lower light intensities intermittence of the lighting is without effect

Warburg's explanation of this effect is that there is probably a reaction forming part of the photosynthetic process which proceeds in the dark to a position of equilibrium , the product of this reaction participates in the photosynthetic reaction, and so will be

present in higher concentration when the dark period is brought to an end than it was at the beginning of the dark period in which it would have been if light had been maintained and it had been continuously used up In low light intensities, on the other hand, the destruction of this substance in the light is not sufficient to shift the position of equilibrium from that which is reached in the dark Consequently the effect of intermittent lighting is not observed

It should be mentioned that Tswett (1911*a*) thought that under the illumination of rotating sectors photosynthetic activity is not related to the total time of illumination, but to this together with an additional period of activity due to phosphorescence Warburg's results could be explained on this basis.

"*Photochemical Induction.*"—Closely allied to the question of intermittent illumination is that of a photochemical induction in photosynthesis The question is whether after a period in the dark a photosynthesising organ on exposure to light proceeds to assimilate at its full intensity, or whether the rate of photosynthesis is low at first and gradually rises to its full value Warburg found with *Chlorella* that there is such a period of photochemical induction. After a period in the dark of 5 minutes the maximum reduction in the rate of photosynthesis occurred on exposure to light The rate was then reduced to 70 or 80 per cent , but no further reduction than this occurred after longer periods of darkening

Experiments performed by Osterhout and Haas (1918*a*) agree somewhat with those of Warburg The experimental plants in this case were *Ulva rigida*, *Enteromorpha*, *Spirogyra*, *Hydrodictyon*, *Potomogeton* and others Plants kept overnight in the dark showed, in an experiment extending over nearly three hours, a steady rise in the rate of photosynthesis from 0 92 to 7 22 during this period, at the end of which the rate is practically steady

A similar rise in the rate of photosynthesis of bean leaves (Canadian Wonder) and *Helianthus annuus* leaves which had been previously kept for several days in the dark, was observed by Spoehr and McGee (1923) They found that the respiration rate ran parallel with the rate of photosynthesis, and conclude that the two processes must be closely connected This is also the view of Warburg, whose theory, elaborated from his experiments on this and the influence of other conditions on photosynthesis, will be discussed in a later chapter

Temperature

It is well known that the rate of many chemical reactions is considerably affected by temperature, being approximately doubled or trebled by a rise in temperature of 10° C The principle stating this relation is known as the Van 't Hoff rule on account of the investigator who first stated it clearly (cf Van 't Hoff, 1898).

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present in higher concentration when the dark period is brought to an end than it was at the beginning of the dark period. It would have been if light had been maintained and it had been continuously used up. In low light intensities, on the other hand, the destruction of this substance in the light is not sufficient to shift the position of equilibrium from that which is reached in the dark. Consequently the effect of intermittent lighting is not observed.

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"*Photochemical Induction.*"—Closely allied to the question of intermittent illumination is that of a photochemical induction in photosynthesis. The question is whether after a period in the dark a photosynthesising organ on exposure to light proceeds to assimilate at its full intensity, or whether the rate of photosynthesis is low at first and gradually rises to its full value. Warburg found with *Chlorella* that there is such a period of photochemical induction. After a period in the dark of 5 minutes the maximum reduction in the rate of photosynthesis occurred on exposure to light. The rate was then reduced to 70 or 80 per cent., but no further reduction than this occurred after longer periods of darkening.

Experiments performed by Osterhout and Haas (1918*a*) agree somewhat with those of Warburg. The experimental plants in this case were *Ulva rigida, Enteromorpha, Spirogyra, Hydrodictyon, Potomogeton* and others. Plants kept overnight in the dark showed, in an experiment extending over nearly three hours, a steady rise in the rate of photosynthesis from 0·92 to 7·22 during this period, at the end of which the rate is practically steady.

A similar rise in the rate of photosynthesis of bean leaves (Canadian Wonder) and *Helianthus annuus* leaves which had been previously kept for several days in the dark, was observed by Spoehr and McGee (1923). They found that the respiration rate ran parallel with the rate of photosynthesis, and conclude that the two processes must be closely connected. This is also the view of Warburg, whose theory, elaborated from his experiments on this and the influence of other conditions on photosynthesis, will be discussed in a later chapter.

Temperature

It is well known that the rate of many chemical reactions is considerably affected by temperature, being approximately doubled or trebled by a rise in temperature of 10° C. The principle stating this relation is known as the Van 't Hoff rule on account of the investigator who first stated it clearly (cf. Van 't Hoff, 1898).

Many plant and animal processes have been shown to obey the rule, if only approximately and within limits (cf Blackman, 1908, Kanitz, 1915) Photochemical reactions, on the other hand, are much less influenced by temperature (cf Plotnikow, 1910, Weigert, 1911, Sheppard, 1914). The temperature coefficient, denoted by the symbol Q_{10}, is the ratio of the rate of the reaction or process at one particular temperature to the rate of the process at a temperature 10° C lower In the case of purely chemical reactions that obey the Van 't Hoff rule Q_{10} is thus in the neighbourhood of 2 to 3, while photochemical reactions are characterised by temperature coefficients which appear rarely to exceed 1 4 and which may be as low as unity, in which case the action is unaffected by temperature Purely physical processes have also for the most part low temperature coefficients in the neighbourhood of 1 2 to 1 3 over the range of temperatures met with in living plants.

As photosynthesis is a process dependent on light, it must be supposed that a photochemical process is involved Determination of the temperature coefficient of the process may therefore be expected to shed some light on the question whether any other process, purely chemical in nature, is involved as well, for in that case the temperature coefficient, providing the photochemical action were not limiting the whole process, might be expected to be of a higher value than that characteristic of photochemical reactions

It has already been mentioned that many processes in organisms have been shown to obey the Van 't Hoff rule approximately and within limits. Thus, Ege and Krogh (1914) have constructed a smooth temperature-metabolism curve giving the relation between temperature and respiration in a fish This curve does not obey the Van 't Hoff rule exactly, as the temperature coefficient is not constant throughout the whole course of the curve, being progressively smaller with rise in temperature Similar curves for the respiration of higher plants have been constructed by Kuijper (1910), who found the Van 't Hoff law followed between 0° and 20°, while above the latter temperature the temperature coefficient fell off rapidly. Similar curves showing the same approximate, but not exact, obedience to the Van 't Hoff rule have been constructed for the rate in growth in length of pea roots by Miss Leitch (1916) Where this divergence from the exact logarithmic relation of the Van 't Hoff rule occurs, it has been suggested (Putter, 1914) that the whole curve is made up of portions of several logarithmic curves with different constants, since the value of Q_{10} is continually changing But it has been justly pointed out by Krogh (1916) that it is not very probable that the Van 't Hoff rule should be followed in vital processes, as these are not as a rule simple chemical reactions, but constitute complex series of reactions taking place in a heterogeneous system. Moreover, if the difference

between the heterogeneous system and the simple system of substances reacting in solution were negligible, the presence of a limiting factor would still be operative Thus, in the case of respiration, the supply of oxygen to the tissues might limit the rate of the reaction at high temperatures to one below that which would be possible at these temperatures if the oxygen pressure in the tissues could be increased Also there is the possibility of secondary actions such as that we have already noticed with high concentrations of carbon dioxide, which have nothing directly to do with the process under consideration That high temperatures may give rise to such complications in regard to plant processes we shall see clearly exemplified in the case of photosynthesis

Attention has already been called to the fact that all methods of measuring photosynthesis give only the value of the " apparent assimilation," which has to be corrected for respiration In determining the influence of temperature on photosynthesis it is particularly necessary to make this correction, since respiration increases rapidly with increase in temperature That respiration in higher plants increases with temperature according to the Van 't Hoff rule has already been noted. In addition to these observations of Kuijper, a temperature coefficient of about 2 was found over the range 5° to 25° by Harder (1915) and Plaetzer (1917) for the respiration of *Cladophora*, while Kurt Noack (1920*b*) found a somewhat lower coefficient, 1·8, for the respiration of thermophilous fungi

The respiration being so influenced by temperature, early work on the effect of temperature on photosynthesis, in which no allowance was made for respiration, need not be considered Nor is much discussion of Kreusler's work (1887, 1888, 1890) on the effect of temperature on photosynthesis called for, since insufficient care appears to have been taken to ensure that the plant material was in a healthy condition Thus, some of his data were obtained with one shoot of *Rubus fruticosus* kept in the assimilating chamber for three weeks and exposed to a different temperature every day It seems clear that under such conditions the health and activity of the shoot would probably be declining from day to day (cf Matthaei, 1904). Kreusler actually found that with increasing temperature photosynthesis increased to a maximum which lay between 15° and 25° C and fell as the temperature was further increased.

Our knowledge of the influence of temperature on photosynthesis is largely due to work done in Blackman's laboratory and described in papers by Miss Matthaei (1904) and Blackman and Matthaei (1905) It has already been noted that the curve between temperature and a vital process will be influenced if a limiting factor should be operative and so prevent the process proceeding at the maximum rate possible at that temperature Blackman recognised that in investigating the effect of temperature on photosynthesis it is of

first importance that no other factor such as light intensity or carbon dioxide concentration be limiting the rate of the process, as in that case the rate of photosynthesis will not be related to the temperature but to the value of the limiting factor

A further complication arises in determining the relation between photosynthesis and temperature in high intensities of illumination This arises from the fact, recognised by Brown and Escombe (1905*a*) as well as by Blackman and Matthaei, that the internal temperature of a leaf will be raised when subjected to a high light intensity. While Brown and Escombe attempted to obtain the internal temperatures of leaves by calculation from a knowledge of other conditions of the leaf, Blackman and Matthaei made direct measurements of the internal temperature electrometrically by means of small copper-constantan thermocouples immersed in the midrib of the leaf It was found in this way that the internal temperature of a leaf subjected to high light intensity might be several degrees above that of the surroundings The values given in the following table indicate the rise in temperature which may occur.

TABLE 19

INFLUENCE OF HIGH LIGHT INTENSITY IN RAISING THE INTERNAL TEMPERATURE OF LEAVES

(Data from Blackman and Matthaei)

Source of light	Relative intensity of light.	Temperature of bath containing leaf chamber in Centigrade degrees	Internal temperature of leaf in Centigrade degrees
Keith high-pressure gas burners . . .	13	11·0	15 0
,, ,,	26	11 0	23 7
,, ,,	45	13 5	30 5
Bright sunlight in July .	—	18 6	22 4–30 7

The leaves used were those of cherry laurel (*Prunus laurocerasus*, var *rotundifolia*) and Jerusalem artichoke (*Helianthus tuberosus*). Leaves were carefully selected and after picking were kept twenty-four hours at fairly constant and the same temperature, with their stalks in water contained in covered beakers exposed to diffuse light This treatment was found to be effective in reducing differences due to different previous history, especially in regard to nutrition and temperature, which are both very important factors in determining the rate of photosynthesis A fresh leaf was employed for each experiment, and the constant gas-stream method used for measuring photosynthesis To reduce water loss from the leaf, which must take place owing to transpiration, the cut end of the leaf-stalk was kept in water throughout the course of the experiment Correction was, of course, made for respiration. In each experiment 800 c c of air containing from 0 8 to 2 8 per cent. of carbon dioxide was passed through the leaf chamber in an hour. The carbon dioxide was always in sufficient concentration not to

act as a limiting factor. The experiment was allowed to run for 1 5 or 2 hours before measurements were taken, after which the absorption of carbon dioxide during consecutive hourly or two-hourly periods was measured. The output of carbon dioxide by the leaf during the same length of time in the dark gave the respiration, which has to be added to the experimentally found values of carbon dioxide absorption to give the true assimilation

The unit of light intensity used in Miss Matthaei's experiments was that of a single incandescent gas burner the front of which was 130 cms from the leaf With this intensity of light photosynthesis was measured at a temperature as low as —6° C As temperature increased the rate of photosynthesis increased up to a temperature of 3° C, above which increase of temperature had no effect on the rate of assimilation, which remained constant even up to 33° C The results are illustrated graphically in Fig 13

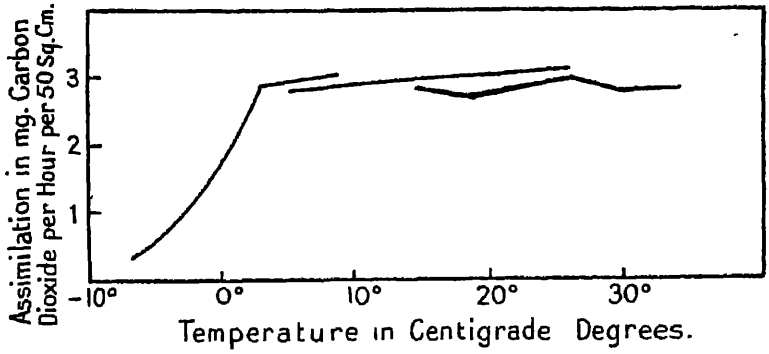

FIG 13 —Graphical representation of the effect of temperature on photosynthesis in cherry laurel with unit intensity of light (After Miss Matthaei)

They strongly suggest that from 3° upwards light is limiting the rate of photosynthesis

If this is the correct explanation of the results obtained with unit intensity of light, doubling the light intensity should result in the first ascending part of the curve becoming much longer, as the limiting action of light would only be operative when photosynthesis was proceeding at double the rate of the maximum possible with unit light intensity This was found to be the case Fig 14 shows graphically the results obtained by Miss Matthaei for the rate of photosynthesis of cherry laurel leaves at different temperatures in light of 1, 2 and 4 units intensity The curves indicate clearly that the higher the temperature the more rapid the rate of photosynthesis, provided a limiting factor is not operative Increase in temperature, on the other hand, produces no effect on the rate of photosynthesis if light inetnsity is a limiting factor

At temperatures below 25° C. the rate of photosynthesis wa found to remain constant hour after hour, as the figures given i Table 20 show The values obtained for the rate of photosynthesi at temperatures between 5° and 25° C. when neither light no

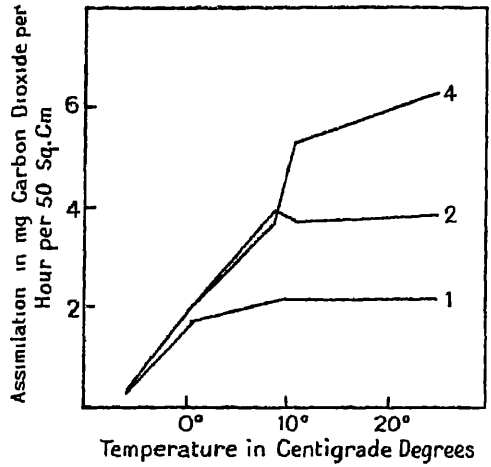

FIG 14 —Graphical representation of the influence of temperature on photosynthesis in cherry laurel under the influence of light of different intensities 1, unit intensity of light, 2, twofold light intensity, 3, fourfold light intensity (After Miss Matthaei)

carbon dioxide concentration is a limiting factor, indicate that between these temperatures the Van 't Hoff rule is obeyed, the temperature coefficient (Q_{10}) being 2·1 Below 5° C the temperature coefficient increases with decreasing temperature This is quite a common phenomenon with life-processes which may be due to some other factor coming into play, but which is not explained

TABLE 20

RATE OF PHOTOSYNTHESIS OF CHERRY LAUREL LEAF AT 8 8° C

(Data from Matthaei)

Light intensity	Time	Real assimilation per 50 sq cms per hour
1	12 30– 2 0 p m	Preliminary
,,	2 0 – 4 0 ,,	0 0023
,,	4 0 – 6 0 ,,	0 00225
2	8 0 – 9 40 ,,	Preliminary
,,	9 40–11 40 ,,	0 0039
,,	11 40 p m –1 40 a m	0 0039
,,	1 40–3 40 a m	0 0038
,,	3 40–5 40 ,,	0 00385
,,	5 40–7 40 ,,	0 00385

Above 25° C, however, a different state of affairs was found. At these higher temperatures there was found to be a continuous falling off in the rate of photosynthesis with time, the higher the temperature the more rapid the decline, while at any one temperature the decline in the rate of photosynthesis was found to be greatest at first, becoming less rapid subsequently An example of an actual series of measurements is given in Table 21.

TABLE 21

RATE OF PHOTOSYNTHESIS OF CHERRY LAUREL LEAF AT 37 5° C

(Data from Matthaei)

Light intensity.	Time.	Real assimilation per 50 sq cms per hour
45	10 30 a m –12 noon	Preliminary
,,	12 0 noon–1 0 p m	0 0237
,,	1 0–2 0 p m	0 0176
,,	2 0–3 0 ,,	0 0139
,,	3 0–4.0 ,,	0 0109

As the measurements of photosynthesis are made over hourly or two-hourly periods, during which time the rate of photosynthesis is continuously declining, it is impossible to measure the initial, that is, the highest possible, rate of photosynthesis at any temperature above 25° C. Blackman estimated the initial rate of photosynthesis at these higher temperatures by two methods Firstly, by assuming the Van 't Hoff rule is obeyed above 25° as well as below it, the initial values of the rate of photosynthesis can be obtained by calculation Secondly, the curve between rate of photosynthesis and time can be plotted for every particular temperature and continued back to the point where the value of time is zero and where the value given of photosynthesis velocity will thus be the initial one The two methods give practically the same results, and in spite of criticisms (Kanitz, 1915, Rahn, 1916, Brown and Heise, 1917*a*) it may be concluded that the rate of photosynthesis of cherry laurel leaves obeys the Van 't Hoff rule, at any rate between limits, the temperature coefficient being not far removed from 2 1

Further experiments by Blackman and Matthaei (1905), in which only natural illumination was used, suggest that leaves of different species may exhibit different temperature coefficients of the rate of photosynthesis Thus, in the case of *Helianthus tuberosus*, the temperature coefficient was found to be about 2 5 (cf Fig 15)

In *Elodea* Blackman and Smith (1911*b*) measured the rate of photosynthesis at 7° and 13° C The values obtained, assuming that photosynthesis in water plants increases logarithmically with temperature, indicate a temperature coefficient (Q_{10}) of 2 05, a value practically identical with that obtained for cherry laurel.

The rate of photosynthesis of *Elodea* at higher temperatures

was investigated by Van Amstel (1916), experiments being performed at temperatures between 24° and 45° C. Above 40° C. temperature had a definitely injurious effect, while below 40° C only two temperatures were employed, namely, 24° and 36 5° C The temperature coefficient of photosynthesis velocity calculated from Van Amstel's results for these temperatures is 1 26, but owing to the lack of uniformity in the material used throughout the research, to the possibility that at the higher temperature light or carbon dioxide concentration might be acting as a limiting factor, and also to the fact that no correction was made for respiration, this low temperature coefficient requires confirmation (cf Smith, 1919). Van Amstel herself thought it extremely improbable that she had determined the rate of photosynthesis at the higher temperatures, but that physical processes had exerted a limiting effect.

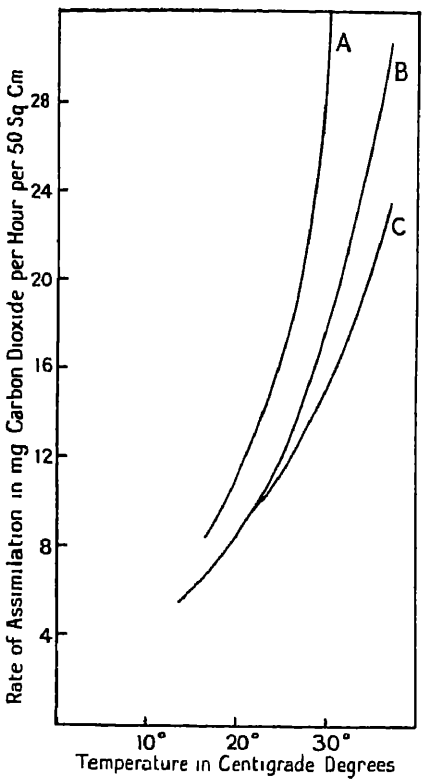

FIG 15.—Curves to show the relation between temperature and photosynthesis of *Helianthus tuberosus* and cherry laurel A, curve of initial assimilation maxima for *Helianthus*, B, the same for cherry laurel, C, curve for assimilation of cherry laurel two hours after the moment of bringing the assimilating leaf to the particular temperature (After Blackman and Matthaei)

The rate of photosynthesis of *Ulva rigida* has been determined at 17° C and 27° C by Osterhout and Haas (1919), the method employed being the colorimetric one depending on the change in alkalinity of seawater during photosynthesis and which has been described earlier (see Chapter V) They find the rate of photosynthesis at 27° is 1 81 times that at 17° C This value for the temperature coefficient is of the same order as those obtained by Blackman and his co-workers Smith (1919) says of this determination by Osterhout and Haas, "Even this figure, however, is probably not high enough, for in the very brief details given of their experiments

in the low CO_2 tension of sea-water they provide no evidence against the natural view that low CO_2 tension, based on slow dissociation, and diffusion were limiting their observed value at 37°[1] to something lower than would have been obtained had temperature been exerting its maximum effect"

Lastly, Warburg (1919) has studied the effect of temperature on the rate of photosynthesis in *Chlorella* Between 5° and 23° he found the temperature coefficient gradually sank from 4 3 to 1 6, but between the temperatures 15° and 25° C he obtained a mean temperature coefficient of about 2 In lower light intensities, however, the rate of photosynthesis is less at higher temperatures than when a strong light intensity is employed, and under these conditions temperature is practically without influence on the rate of photosynthesis over the temperature range 15° to 25°. This is to be expected if light intensity is a limiting factor

From this review of the work done on the influence of temperature on photosynthesis it appears that provided neither carbon dioxide nor light intensity is a limiting factor, the rate of photosynthesis obeys the Van 't Hoff rule, at any rate between about 5° C. and 25° C, the rate of photosynthesis being about doubled for an increase in temperature of 10° C Below 5° C the temperature coefficient increases with decreasing temperature, while at high temperatures, in the case of cherry laurel those above 25° C, matters are complicated by the presence of a "time factor," the rate of photosynthesis falling off with time If, however, the initial values of photosynthesis at these higher temperatures are obtained by extrapolation from those obtained after photosynthesis has proceeded for various periods, it would appear that the Van 't Hoff rule is obeyed at these higher temperatures

In a critical review of the work on the influence of temperature on photosynthesis, Brown and Heise (1917*a*) hold that the available data suggest a temperature coefficient of photosynthesis velocity more in the neighbourhood of those characteristic of photochemical reactions The work of Prjanischnikow (1876), Kreusler, Lubimenko (1906) and Van Amstel, which is cited in support of the low temperature coefficient, cannot be regarded as giving trustworthy data for calculation of temperature coefficients, while there is certainly no less reason for accepting the temperature coefficients proposed by Matthaei and Blackman and Smith from their own work than for accepting the lower coefficients proposed by Brown and Heise from a consideration of the data of these workers Moreover, since the publication of Brown and Heise's criticism, the work of Osterhout and Haas and of Warburg has added further evidence of the obedience of photosynthesis to the Van 't Hoff rule [2]

It is interesting to know the lower limit of temperature at

[1] Obviously a misprint for 27°

[2] For very recent contributions on the influence of temperature on photosynthesis, see Yabusoe (1924), Lundegårdh (1924*b*) and Harder (1924)

which photosynthesis takes place It has already been mentioned that Matthaei observed definite photosynthesis in cherry laurel at —6° C Henrici (1921) has observed photosynthesis in lichens at —20° C, in alpine shade plants at —16° C and in sun plants at temperatures which are higher but still below 0° C. It is possible, as Ewart (1896) asserts, that in such cases the cessation of photosynthesis is due to withdrawal of water from the protoplasm to form crystals of solid ice Ewart further states that in warm temperate, sub-tropical and water plants evolution of oxygen ceases between 0° and 2° C., while in tropical plants between 4° and 8° C Cloez and Gratiolet (1850) found that assimilation commenced in *Potamogeton, Ceratophyllum* and *Conferva* between 10° and 15° C, and in *Vallisneria* above 6° C., but Ewart suggests that by the use of the bacterium method evolution of oxygen can be detected several degrees below these temperatures

Wurmser and Jacquot (1923) found that subjecting various marine algæ, *Ulva lactuca, Codium tomentosum, Rhodymenia palmata, Laminaria saccharina, L digitata* and *Iridea edulis* to temperatures between 36° C and 45° C for two minutes, and in the case of *Ulva lactuca* to periods varying from 1 to 15 minutes, lessened the photosynthetic activity when the plants were returned to sea-water at 16° C. It was found that with increase in temperature and in the time of exposure to the high temperature the depressing effect of exposure to the high temperature became more marked, while photosynthesis was completely suppressed by exposure to a temperature below that fatal to the plant concerned Wurmser and Jacquot suppose that the inhibition of photosynthetic activity resulting from exposure to high temperature is due to a modification of the state of the cell colloids which has the effect of throwing out of action the mechanism responsible for the reduction of carbon dioxide in the assimilating cell But with Wurmser's views on the photosynthetic mechanism we shall deal in a later chapter

Water Content

Water is required equally with carbon dioxide for the production of carbohydrates in the green leaf, but it is scarcely to be expected that the variations in water content which are likely to be met with in leaf cells are relatively great enough to affect the rate of photosynthesis at all considerably Nevertheless, it has been known for some time that withdrawal of water from the leaf does depress the rate of photosynthesis Thus Kreusler (1885) found that the rate of photosynthesis of cut branches exposed to strong insolation rapidly declined, while Nagamatz (1887) found by means of Sachs's iodine test that wilted leaves of *Atropa Belladonna, Sambucus nigra, Beta trigyna, Aquilegia glauca, Vitis Labrusca* and *Dipsacus laciniatus* had no power of forming starch More exact observations have been made by Thoday (1910) with an improved

form of the half-leaf method In Table 22 are shown the average rates of assimilation of leaves of *Helianthus annuus* exposed in the open air in July and August to sunlight The relation between rate of photosynthesis and degree of turgidity of the leaves is undoubted. Confirmatory results, though not so strikingly regular on account of the high degree of asymmetry of the leaves, were obtained with *Catalpa bignonioides*

TABLE 22

RATE OF PHOTOSYNTHESIS OF LEAVES OF *Helianthus annuus* OF DIFFERENT DEGREES OF TURGIDITY

(Data from Thoday)

Condition of leaves	Average rate of photosynthesis in mg per sq decimetre per hour
Turgid	16 1
Moderately turgid, occasionally rather limp	12·5
Limp	8 5
Limp to flaccid	5·3
Quite flaccid from beginning	1 6

The lowered rate of photosynthesis with lowered water content of the leaves, is ascribed in many cases to closure of the stomata with water loss from the leaves This was Sachs's opinion expressed in a footnote to Nagamatz's paper cited above, while the same view is held by Thoday, who followed the closure of the stomata by means of the horn hygroscope of F Darwin (1898) Supporting this view is the fact that in plants without stomata, such as mosses and lichens, photosynthesis is much less depressed by loss of water than is the case with foliage leaves of higher plants (Bastit, 1891 , Jumelle, 1892) Also Klebs (1888), in observations on the physiology of algæ, concluded that *Zygnema* could assimilate when the cells were plasmolysed and contained in consequence considerably less water than normally. Kny (1897) obtained similar results. On the other hand, Treboux (1903) and Pantanelli (1903) have observed a decline in the rate of photosynthesis of water plants with decreasing turgidity of the cells, even before the first appearance of plasmolysis, and in such plants closure of stomata cannot be invoked to explain the result

Further observations on the effect of water shortage on photosynthesis have recently been described by Iljin (1923) Measurements were made of the water content of the leaves, the degree of opening of the stomata, and the rate of photosynthesis The stomatal aperture was determined by means of the porometer and the rate of photosynthesis was determined eudiometrically Results similar to those of Thoday were obtained After complete closure of the stomata further withdrawal of water further reduced the rate of photosynthesis, but no proportionality appeared between photosynthesis velocity and water content

By means of Sachs's iodine test, Dastur (1924) has shown that in ageing leaves of *Abutilon asiaticum, Ricinus communis, Carica papaya*

and a number of other species, a loss of photosynthetic activity occurs first in the mesophyll cells of the margin and intravascular regions of the leaf, that is, in the cells most remote from the vascular bundles and therefore from the water supply The "photosynthetic decay" then spreads inwards towards the centre and main veins In further work, unpublished at the time of writing, the same author has shown, by determinations of the water content and photosynthetic activity of leaves of the same species in different stages of development, that there is a direct correlation between water content and rate of photosynthesis, the latter declining as the water content diminishes as the leaves approach their stage of senescence.

The Wave-Length of the Incident Light

In the discussion, earlier in this chapter, of the influence of light on photosynthesis, it was the effect of varying intensities of the white light obtained from the sun or from artificial sources of illumination such as incandescent gas or electric lamps that was considered Such light is, of course, composed of rays of various wave-lengths corresponding to the various colours of the normal spectrum and varying from about 770$\mu\mu$ in the extreme red to about 390$\mu\mu$ in the extreme violet. In addition, invisible rays of longer wave-length than the extreme visible red (the infra-red rays) and of shorter wave-length than the extreme violet of the spectrum (the ultra-violet rays) may be, and generally are, present in the light obtained from such sources of illumination [1] It is obviously part of our business to inquire whether all the rays that go to make up white light are equally effective in photosynthesis, or whether waves of any particular wave-length are exclusively, or particularly, concerned in supplying energy for the photosynthetic process

This particular problem has excited the interest of plant physiologists from very early times in the history of photosynthesis Senebier (1788) thought the rays of the blue-violet part of the

[1] It is tacitly assumed in experiments on the influence of light intensity on photosynthesis that a reduction in light intensity involves a proportionate reduction in the intensity of radiation of all the constituents of white light This may not necessarily be the case Thus, if the reduction in light intensity is brought about by increasing the distance between source of light and assimilating material, certain wave-lengths may be absorbed by the intervening material For example, glass absorbs ultra-violet radiations while water absorbs infra-red Now, the law relating amount of absorption with thickness of the absorbing medium is not one of direct proportionality, but is represented by the equation $a = i(1 - e^{-\alpha d})$, where a is the decrease in intensity in the distance d, i is the intensity of the light when it enters the absorbing medium, and α is the absorption coefficient Hence, doubling the distance between source of light and assimilating material may, approximately enough, reduce the intensity of illumination at the assimilating surface to one-fourth according to the law of inverse squares, but the actual composition of the light will not necessarily be exactly the same in the two cases.

spectrum were those concerned, a view adopted by Dumas and Boussingault (1841). Gilby (1821) examined photosynthesis in red and blue light, but a more systematic attack on the problem was made by Daubeny (1836), who illuminated parts of plants (land plants with the exception of *Laminaria digitata*) in water saturated with carbon dioxide under glass or liquid screens of different colours and determined the gas produced Draper (1843, 1844) performed similar experiments, using the colours of the spectrum obtained with a prism as well as those obtained with the use of screens The results of both workers indicated a maximum assimilation in yellow light, that is, in the brightest region of the spectrum, while little or no assimilation was observable either in the extreme red or in the blue-violet region That photosynthesis was most active in the yellow rays of the spectrum was also the conclusion of Hunt (1848), Cloez and Gratiolet (1850), Sachs (1864*b*), Pfeffer (1871) and N J C. Muller (1872) Pfeffer, like Daubeny and Draper, considered that the photosynthetic activity corresponded to a great extent with the brightness of the light, that is, with the intensity of the illumination

It was suggested by Lommel (1871) that the rays most strongly absorbed by chlorophyll, that is, those in the red part of the spectrum between the B and C lines, are those most effective in photosynthesis, and in the experiments of Timiriazeff (1869, 1877, 1883, 1889) and Reinke (1884*a*, 1885*a*, *b*, 1893) in which photosynthesis in light of different wave-lengths was determined by eudiometric and the gas-bubbling methods respectively, the most intensive rate of assimilation was found in this region Engelmann (1882*a*, 1884) investigated the problem by projecting a spectrum on a green algal filament and observing the relative intensities of photosynthesis in different regions of the spectrum by the bacteria method, the accumulation of bacteria in different regions of the spectrum being taken as a measure of oxygen output and hence of photosynthesis In this way Engelmann found a secondary maximum of photosynthetic activity in the blue-violet part of the spectrum as well as the primary maximum in the red observed by Timiriazeff and Reinke The same method has been used subsequently with the leaves of flowering plants Timiriazeff (1890, 1903) projected a spectrum on a destarched leaf of *Hydrangea* and found that a significant formation of starch took place in the red while the amount produced decreased progressively towards the violet end of the spectrum, there being hardly any production of starch in the blue and violet More recently Ursprung (1917) has criticised Timiriazeff's experimental arrangement, and has repeated his experiments with *Phaseolus multiflorus* and a few other plants, using a number of different sources of light In his first experiments Ursprung found that with all sources of light no starch was formed in the infra-red, the limit at this end of the spectrum being at about the line A, while the maximum starch formation was

found to occur quite near this line, namely, between the lines *a* and B Starch formation was found to occur throughout the whole visible spectrum and in the ultra-violet, the limit at this end of the spectrum being at about 342$\mu\mu$ in sunlight and at about 330$\mu\mu$ in the light of the electric arc In the latter secondary maxima were observed in the violet and ultra-violet at about 420$\mu\mu$ and 388$\mu\mu$, the former not always occurring These maxima which do not occur in sunlight, appear to be related to corresponding bands in the spectrum of the arc

Subsequently Ursprung (1918*a*) was able to observe slight starch formation in leaves of *Phaseolus vulgaris* exposed for 40 hours to the infra-red rays from a 2500 candle-power Osram projection lamp Visible rays were removed by filtering the light through either a layer of a concentrated solution of iodine in carbon disulphide 9 cm thick or an ebonite plate 1 1 mm in thickness. Ursprung thinks that limitation of starch formation in the infra-red may be due to closure of the stomata, so that actually carbon dioxide concentration in the assimilating cells is a limiting factor

The range of wave-length (330–760$\mu\mu$ or beyond) over which Ursprung found starch formation takes place is wider than that over which Reinke by the bubbling method and Engelmann by the bacteria method found evolution of oxygen, the range found by these workers being about 400–750$\mu\mu$ and 397–718$\mu\mu$ respectively Previously Bonnier and Mangin (1886*a*) had recorded weak photosynthesis in ultra-violet radiation, but it would appear doubtful whether the filters they used excluded all other radiation (cf. Ursprung, 1917)

While the work already considered enables us to fix with some degree of certainty the range of wave-lengths in which photosynthesis can proceed, it does not provide us with any reliable data in regard to the effectiveness of light of different wave-lengths for photosynthesis. For it is clear from the results of the investigations on the influence of light intensity on photosynthesis which have been dealt with in an earlier section of this chapter that if the effectiveness of lights of different wave-lengths is to be examined it is essential that photosynthesis should be compared in such different-coloured lights *of the same intensity*, while it is also essential that no factor other than intensity of illumination is limiting the process In the investigations on photosynthesis in different coloured lights so far considered these conditions were not realised Certain investigators, for example, Timiriazeff (1893) and Richter (1902), realised that the greater effectiveness of red light than of blue-violet is to be explained by the different intensity of illumination, that is, the different quantity of energy, in the two regions (cf. Palladin, 1911)

An attempt to determine whether red, green and blue light of the same energy would enable photosynthesis to take place at the same rate under otherwise similar conditions was made by

Kniep and Minder (1909) Radiant energy was measured by means of a Rubens thermopile and photosynthesis by the bubble-counting method, the plant employed being *Elodea* Light of the various colours was obtained by means of filters The red filter was a red glass screen which allowed all rays from 620$\mu\mu$ to infra-red to pass through it, as well as a little light of wave-length 608$\mu\mu$. The blue filter transmitted light of all wave-lengths between 523 8$\mu\mu$ and about 340$\mu\mu$ in the ultra-violet, while the green filter consisted of a solution of potassium chromate and ammoniacal copper oxide which allowed through light of wave-lengths between 524$\mu\mu$ and 512$\mu\mu$ Sunlight was employed as source of illumination, all experiments being performed on cloudless days at Naples between 11 a.m and 2 30 p m , during which time both the intensity of light and distribution of energy in the solar spectrum remain practically constant.

Kniep and Minder concluded from their experiments that red and blue lights of the same intensity bring about the same rate of photosynthesis, while no assimilation takes place in green light. For a number of reasons, however, the work of Kniep and Minder is open to criticism Thus, in order to expose the plant material to the same intensity of illumination in all cases, a series of screens of various substances such as water and solutions of copper sulphate and potassium dichromate were introduced between the source of light and the assimilating plant material, so that the actual composition of the light employed was probably different from that which passed through the red and blue screens used singly Further, it is not clear that light intensity was the limiting factor in the experiments, although, as the absolute intensity of the energy incident on the plants was of the order of only 0 005 gram-calorie per square centimetre per minute, it very probably was so But the chief objection to the work of Kniep and Minder is to the method used to measure the rate of photosynthesis The crude bubble-counting method, as has been indicated in an earlier chapter, abounds in sources of error, as, indeed, Kniep himself subsequently recognised (Kniep, 1915). Further, Ursprung (1918*a*) is of opinion that the infra-red rays passed through both the green and blue filters as well as through the red, and as the infra-red rays of the solar spectrum account for 60 to 80 per cent of the total energy of the solar spectrum, the energy measurements made by the thermopile may appear very much larger than the energy values of the photosynthetically active rays

Ursprung himself (1918*a*) made observations on the relation between wave-length and starch formation in leaves of potted plants of *Phaseolus vulgaris* An electric arc lamp was used as a source of light, and the different wave-lengths were obtained by means of a prism. Light intensity was measured by means of a linear vacuum thermopile. A series of experiments were made in each of which light of the same intensity but of different wave-

length passed through two slits, symmetrically disposed on the two sides of the midrib of the leaf, on to the surface of the leaf After exposure to light for 2 to 7 hours the leaves were tested with iodine and the degree of blackening on the two sides of the leaf compared and referred to an arbitrary scale A curve exhibiting the relation between wave-length of light and starch formation was constructed from the data so obtained At the extreme red end of the spectrum starch formation was found to be practically nil, but with decreasing wave-length the formation of starch rapidly increased, reaching a maximum at about the C line (about 656$\mu\mu$), and decreasing slowly with decreasing wave-length up to the violet end of the spectrum, but with a series of secondary maxima at about 620$\mu\mu$, 589$\mu\mu$ (the D lines), 532 $\mu\mu$, 488$\mu\mu$ (near the F line) and 431$\mu\mu$ (the G line)

Lubimenko (1923) has more recently compared the rates of photosynthesis in the red (760–600$\mu\mu$) and blue (480–400$\mu\mu$) rays of sunlight, the intensities of red and blue lights being in the proportion of 100 : 85, and concludes that in eight species employed photosynthesis, at 20° C and in 9 to 11 per cent. carbon dioxide, is more active in red than in blue light, although the ratio of the rates of assimilation in red and blue lights varies with the time and species. Only in species normally living in diffuse light of low intensity such as *Aspidistra elatior* and *Hedera Helix* does the assimilatory activity in blue light equal or exceed that in red light

It appears clear that photosynthesis is not equal in light of different wave-lengths but of the same energy, nor should we expect this to be so The leaf absorbs some rays to a much greater extent than others, and it is to be expected that in light of the same intensity photosynthesis will be more rapid when the light is composed of wave-lengths which are absorbed than of those which are largely transmitted. We are here clearly brought into immediate contact with the problems of the energy relations of the photosynthetic process, and consequently a further discussion of the relation of assimilation to the absorption of radiation of different wave-lengths will be postponed until the problems of the energy relations of the green assimilating organ are discussed. As far as the influence of the wave-length of the incident light on the rate of photosynthesis is concerned we may conclude that with equal intensity of incident light photosynthesis is much influenced by wave-length, the evidence going to show that it is greatest in the red, between the B and C lines, and, on the whole, least in the blue-violet That Kniep and Minder obtained no evidence of photosynthesis in the green Ursprung ascribes possibly to the fact that the green filter used by the former workers let through infra-red rays, with the result that the energy measured by the thermopile was chiefly due to the practically ineffective infra-red, so that the intensity of green light was extremely small

In determining the effect of the wave-length of light on the rate

of photosynthesis a difficulty arises even if the intensity of the incident light is maintained the same with the different radiations This difficulty arises on account of the different degrees of absorption of the different-coloured lights by the assimilating cells. Thus Pfeffer (1900) said, " A correct knowledge of the assimilatory effect of the different regions of the spectrum can only be obtained by determining the amounts of carbon dioxide decomposed by the superficial chloroplastids, for the more deeply seated ones receive light of altogether different composition to that which falls upon the outer surface." The so-called primary curve of assimilation which Engelmann obtained with *Cladophora* and the use of the bacterium method and a direct sun-spectrum is supposed to be that given by the superficial chloroplastids But, of course, the intensity of incident illumination is not uniform throughout the spectrum. If radiations of different wave-lengths but of the same intensity fall on a thick leaf, some will be absorbed much more than others in the superficial layers, so that in lower layers of the leaf there will be proportionally less and less of the more absorbed rays available. Consequently, the relative rates of photosynthesis of assimilating organs in different-coloured lights will depend on the thickness of the organ, the thicker the organ the less the differences in the rates of photosynthesis in the different-coloured lights It is thus impossible to find values for the relative rates of photosynthesis in lights of different wave-lengths which will hold in all cases On the other hand, it might be possible to find a general relation between the quantity of energy *absorbed* from light of different wave-lengths and the rate of photosynthesis This question will be dealt with later when the energy relations in photosynthesis are considered

Deficiency of Nutrient Salts

The influence of a deficiency of nutrient salts on photosynthesis has been investigated by Briggs (1922*b*) Plants of *Phaseolus vulgaris* were grown in culture solutions devoid of one of the elements necessary for normal growth, namely, potassium, magnesium, iron or phosphorus. In each case the photosynthetic activity of the leaves was markedly less than that of leaves of plants grown on a complete nutrient solution, and the same depression resulted whether the external conditions were such that light or temperature was limiting

To explain this result Briggs supposes that the " reactive surface " of the chloroplasts is reduced in the plants supplied with insufficient nutrient salt For a reduction in the extent of the reactive surface means a reduction in the surface over which a chemical reaction, the controlling action when temperature is limiting, will proceed, and equally, a change in reactive surface means a change in the extent of the light absorbing surface, so that

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when light is the limiting factor reduction of the reactive surface will also result in decrease in rate of photosynthesis

In support of this view can be cited the record of Gris (1857) that chlorotic plants, and plants grown in absence of light, contain smaller chloroplasts than normal plants A Meyer (1918*a*) records a progressive decrease in size of the chloroplasts as the leaves of *Tropæolum majus* grow old and turn from green to yellow, and, as we shall see, the photosynthetic activity relative to the quantity of chlorophyll is often low in autumn leaves (see p 131).

The "reactive surface" of the chloroplast is not necessarily identical with the actual visible surface of the chloroplast, for it has to be remembered that we probably have in the chloroplast a heterogeneous system, and the "internal" surface may be large and may be influenced by conditions

A point of interest in Briggs's research is that he found the photosynthetic activity of the leaves of a plant grown in a complete nutrient solution less than that of the leaves of a plant of the same species grown in soil, but otherwise under the same external conditions. This result is not fully explained, but it may be due to a shortage of the supply of some essential nutrient element

Stoklasa and Matoušek (1916) and their co-workers believe that potassium plays an important part in the fundamental processes of photosynthesis, and among their arguments in support of their view they cite the results of experiments with sugar beet, in which they found that the leaves of plants growing in a complete nutrient solution assimilated more than three times as rapidly as plants growing in a similar solution without potassium But as Briggs has found the same depression in the rate of photosynthesis occurs when magnesium, phosphorus or iron is omitted from the culture solution, clearly these particular experiments of Stoklasa and Matoušek cannot be used in favour of the view of the all-importance of potassium as compared with other nutrient elements

Since magnesium enters into the composition of chlorophyll, it is to be expected that a deficiency of this element may result in reduced photosynthetic activity on account of a poor development of chlorophyll (cf Mameli, 1912). It has been suggested by André (1916) that photosynthetic activity depends on the ratio of "organic" to "residual" magnesium (and also on the ratio of organic to residual phosphorus, which agrees with the magnesium ratio) in the leaves, organic magnesium being that part of the magnesium extractable with hot ether and alcohol, the residual magnesium being the insoluble part left

Osmotic Pressure of the Medium

If the osmotic pressure of the medium surrounding a water plant is great enough to produce plasmolysis the question resolves itself, as far as photosynthesis is concerned, into that of deficient

water supply, and the same is probably the case when the osmotic pressure of the external medium is high, but not sufficiently so to produce plasmolysis The work of Klebs, Kny and others on this question has already been mentioned in the previous section of this chapter dealing with the effect of water supply on photosynthesis.

The work of Legendre (1921) and Fromageot (1923*a*) appears, however, to require special mention here The former concluded, from observations of *Ulva lactuca* and *Fucus serratus*, that, with decreasing density of sea-water, the rate of photosynthesis increases until a maximum is reached when the density is 1 010, the rate of photosynthesis decreasing with further decrease in the density of the sea-water. Legendre does not regard this, apparently, as related to osmotic pressure in any direct way, but he thinks the content of carbonate and bicarbonate may be of importance in determining the observed facts

Fromageot also determined the rate of photosynthesis of *Ulva lactuca* in sea-water of varying concentration, the density varying from 0 03 to 4 20. He also found an optimum density for photosynthesis, but at 1·94, and he concludes that there is an optimum osmotic pressure of the medium for photosynthesis.

Oxygen

A number of observations are on record which suggest that photosynthesis can only proceed in presence of oxygen Boussingault (1865, 1868) examined the action of hydrogen, nitrogen, carbon dioxide and methane on an assimilating plant in an atmosphere free from oxygen Under such conditions he found that plants in the dark pass into a condition of asphyxia, and in light they no longer have power to decompose carbon dioxide although in appearance they are normal. Pringsheim (1887) also found that in an atmosphere of hydrogen and carbon dioxide *Chara* cells lose their power of assimilating carbon dioxide, a result attributed by him to absence of oxygen. But if oxygen is necessary for photosynthesis to commence, only a relatively very low partial pressure of this appears to be necessary (cf Boehm, 1873), and Friedel (1900, 1902) found that although assimilation diminished with the total pressure it was otherwise unaffected over a wide range of oxygen concentrations, and, in the case of *Euonymus japonicus*, proceeded when initially no oxygen was present (Friedel, 1905)

The question has more recently been re-examined by Willstatter and Stoll (1918), who criticise some of the earlier experiments on the ground that treatment with hydrogen, for which the plant is not adapted, may disturb the protoplasm and so the assimilatory apparatus Consequently Willstatter and Stoll employed an oxygen-free atmosphere consisting of a mixture of nitrogen and carbon dioxide The experimental plants used were *Pelargonium* (two species), *Cyclamen europeum*, *Polytrichum juniperinum* and *Leuco-*

bryum glaucum Reduction of the partial pressure of oxygen to one-hundredth of that of the atmosphere was found not to affect photosynthesis, but in an atmosphere completely devoid of oxygen assimilation decreased more or less rapidly according to the species. Thus the leaves of *Pelargonium* were quite incapable of photosynthesis after exposure to a current of oxygen-free gas for two hours before illumination Even after addition of oxygen they were unable to assimilate *Cyclamen europeum* and *Polytrichum juniperinum*, on the other hand, showed much more resistance to absence of oxygen. The results with 20 grams of leaves of *Cyclamen europeum* are shown in the following table and indicate that although exposure to an oxygen-free medium reduces photosynthesis more and more the longer the exposure, yet even after 15 hours there is still slight photosynthesis, and this increases with continued exposure to light Similar results were obtained with *Polytrichum*

TABLE 23

EFFECT OF ABSENCE OF OXYGEN ON PHOTOSYNTHESIS OF LEAVES OF *Cyclamen europeum*

(Data from Willstätter and Stoll)

Length of exposure in hours to oxygen free gas before illumination	Rate of photosynthesis in grams of carbon dioxide absorbed per hour	
	In first 20 minutes of illumination.	After 1 to 1 5 hours illumination
0 25	0 12	0 157
1 0	—	0 127
2 0	0 07	0 106
15 0	0 0018	0 018

The different behaviour of *Pelargonium* and *Cyclamen* is explained by Willstatter and Stoll on the ground that oxygen removal takes place in two stages, the first consisting in the withdrawal of free oxygen, and the second in the withdrawal of loosely bound oxygen from the cells So long as the second phase has not set in the leaves retain their power of assimilation, and it is resistance to the second phase that constitutes the difference between *Cyclamen* and *Pelargonium*, the former being considerably more resistant than the latter.

Spoehr and McGee (1923) point out that leaves that have been kept in the dark so that they have a low carbohydrate content withstand absence of oxygen less than those with a higher carbohydrate content. This, they hold, supports their view that photosynthesis is dependent on respiration, a view which appears to have been in the mind of Boussingault. This question will be discussed later

Warburg (1919) examined the effect of oxygen pressures between 0·02 and 1 atmosphere on the rate of photosynthesis of *Chlorella* He found that in high light intensities the rate of photosynthesis

sank from 81 to 55 as the oxygen pressure rose from 0·02 to 1 atmosphere The rates of photosynthesis are given in arbitrary units The rate of respiration was found to be unaffected, nor is the rate of photosynthesis influenced by oxygen pressure in low light intensities Warburg suggests two possible explanations of his results Either in high light intensities the products of assimilation are formed in high concentration and are oxidised back by the oxygen before they pass into a stable form, or the oxygen reacts with a substance supposed by Warburg to be formed by photochemical action on the chlorophyll molecule, a substance called by him the "photochemical primary product," which is supposed to participate in the photosynthetic process

Various Substances

Anæsthetics —The influence of ether and chloroform on photosynthesis appears always to be depressing Claude Bernard (1878) found that by treatment with chloroform, water plants ceased to assimilate while they continued to respire His experiments were repeated by Schwarz (1881), who found that sooner or later the rate of evolution of oxygen from *Elodea* decreased in presence of ether or chloroform, but that at the same time the plants were injured, so that with complete cessation of photosynthesis the plants were quite dead In a saturated aqueous solution of ether or chloroform the decline in the rate of assimilation is very rapid, the latter stopping completely in a few minutes Pringsheim (1887) came to a similar conclusion from observations with the bacterium method Bonnier and Mangin (1886*b*), however, confirmed Bernard's conclusion, for they found, by the use of the eudiometric method, that by using a definite proportion of ether in the plant chamber photosynthesis could be completely stopped while respiration proceeded at the normal rate (cf also Detmer, 1882 , Jumelle, 1892) Ewart (1896) obtained similar results with the bacterium method He found that short exposure of certain mosses, *Bryum cæspitosum*, *Orthotrichum affine* and *Dicranum scoparium*, to a strong concentration of ether, seems to be always followed by the death of the cell With lower concentrations of ether, however, it was found possible to bring about a temporary cessation of photosynthesis with subsequent recovery He later (1898*c*) confirmed Bernard's results, using *Elodea*

It appears, then, that by adjustment of the concentration of ether or chloroform, and of the time of action of the anæsthetic, either of these substances can bring about a diminution or cessation of photosynthetic activity, and that subsequent recovery is possible There appears to be no sound evidence of anæsthetics, even in very low concentration, ever acting as a stimulus to photosynthesis The conclusion of Kegel (1905) to this effect was shown by Schroeder (1908) to be due to errors of experimentation, and Miss Irving (1911)

found that very small doses of chloroform were sufficient to arrest photosynthesis in an illuminated leaf of cherry laurel, and that if the chloroform was allowed to act in a very low concentration for only a short period only partial recovery of assimilatory activity took place

Warburg (1919) similarly found that phenylurethane, as well as methylurethane and its homologues, produced a retardation of photosynthesis of *Chlorella* in very low concentrations of the anæsthetic, even in concentrations in which respiration is stimulated In higher concentrations both respiration and photosynthesis are retarded

Antipyrin—Ewart (1896) found that antipyrin acted in much the same way on the assimilation of *Elodea canadensis* and *Chara fragilis* as ether and chloroform on the mosses he examined. Assimilation could be reduced or even stopped, and if exposure was not too long nor the concentration of antipyrin too high, recovery of assimilatory activity was observed Jacobi (1899) also recorded the unfavourable action of antipyrin on assimilation.

Acids—A number of observations are on record indicating that acids in dilute concentration increase the rate of assimilation as observed by the evolution of bubbles by submerged plants Thus Stutzer (1878) found that in absence of carbon dioxide, evolution of oxygen from submerged plants such as *Ceratophyllum* can be induced by 0 025 per cent oxalic acid, as well as by oxalates and potassium hydrogen tartrate in concentrations of from 0·025 to 0 05 per cent. A similar effect on the assimilation of *Ceratophyllum* was observed by Adolph Mayer (1878) as a result of adding calcium hydrogen malate to the water in which the plant was growing Stutzer explained his result on the ground that the oxalic acid or oxalates supplied were oxidised to carbon dioxide by a respiratory process, so that a fresh source of carbon dioxide became available for assimilation. Mayer, on the other hand, appeared to suppose that the acid malate acted on carbonate or bicarbonate bound in the plant, with production of a further supply of carbonic acid

Ewart (1896) recorded an action of phosphoric acid on assimilation similar to that he observed with anæsthetics and antipyrin, finding no suggestion of any stimulatory action

While Wieler and Hartleb (1900) could not detect any favourable influence of traces of hydrochloric acid on assimilation, Treboux (1903), as a result of numerous experiments with submerged water plants and various acids, showed conclusively that in the cases he examined dilute acids certainly brought about a very definite increase in the rate of assimilation

As an example of Treboux's results the following may be cited Shoots of *Elodea* in water containing from 0 1 to 0 3 per cent. carbon dioxide increased their rate of assimilation as measured by the bubble-counting method, in the proportion of, for example, 26 to 46, when the water was acidified with 0 0001 M hydrochloric

acid. Acidification with 0·00001 M nitric acid increased the rate of bubbling 100 per cent, and 0·00098 per cent. sulphuric acid brought about the same relative increase. In increasing the concentration of hydrochloric acid from zero to 0·0004 M there was a progressive increase in the rate of bubble evolution. Neutral chlorides, sulphates and nitrates do not induce any such increase in the rate of bubbling, so it may be concluded that the action is due to the hydrogen ion and not to the kation.

Treboux appears to have been satisfied by regarding the action of acid as a stimulus to the photosynthetic process. This attitude has been criticised by Willstatter and Stoll (1918), who regard it as very unlikely that the action of acid is in any way comparable to an enzyme action influencing directly the assimilatory process. They suggest that a more likely explanation is that part of the carbon dioxide adsorbed to substances in the plant is displaced by the acid so that a development of carbon dioxide is the result, giving rise either to separation of gas or to a temporarily higher concentration of carbonic acid in the chloroplast. The suggestion made in the work cited and earlier (1915*c*), that the bubbles evolved on addition of acid contain a more or less large proportion of carbon dioxide, appears to be without foundation, for Treboux found that his control experiments either gave no bubbles at all in the dark, or they gave a smaller evolution of gas, for which he allowed in calculating the rate of assimilation.

The question has been thoroughly re-investigated by Wilmott (1921), using the improved technique described in an earlier chapter, and by Benecke (1921). The results and conclusions of these two completely independent writers are in close agreement. Treboux's results are more or less confirmed. Thus Wilmott found that *Elodea* collected from a stream near Cambridge gave off bubbles about twice as fast in water containing 0·005 M hydrochloric acid in addition to one per cent. carbon dioxide (expressed as volumes of carbon dioxide at 0° C. and 760 mm. pressure) as in the same solution without the acid. Similar results were obtained by Benecke with *Ceratophyllum demersum* and *Potamogeton densus* as well as with *Elodea* when the solutions were acidified with dilute sulphuric acid. Similar results were obtained when dilute solutions of potassium bicarbonate were used as source of carbon dioxide. In experiments with potassium bicarbonate as source of carbon dioxide Benecke observed a more rapid formation of starch in *Elodea* in acidulated solutions. By means of actual gas analysis he further confirmed the increase in the rate of assimilation produced by dilute acid.

To determine whether the action of acids in increasing the rate of assimilation might be due to the acid setting free carbonic acid from the plant, as suggested by Mayer, Wilmott made experiments with *Elodea* which had been growing for months in an open wooden tub, the water contained in which was largely rain-water. Such

material shows no response to acidification of the water as noted by Treboux, whereas in the case of *Elodea* obtained from a chalky stream, the presence of dilute acid, as we have seen, greatly influences the rate of bubbling Wilmott therefore concluded that *Elodea* in chalky streams becomes impregnated at its surface with calcium carbonate, though not to such an extent that the effect is visible to the eye, and that after growing for a time in soft water the shoot loses its calcification The increase in assimilation brought about by acidification is thus simply due to the action of acid in releasing carbon dioxide from the carbonate, so that the concentration of the carbon dioxide is increased and the rate of assimilation also, provided that carbon dioxide is a limiting factor

Additional evidence in support of this view is forthcoming from the fact that when light, and not carbon dioxide, is the limiting factor, acidification of the external solution produces no effect on the rate of assimilation

Further evidence comes from the work of Benecke This worker points out three possible explanations of the acid effect, namely, (1) the acid may act on the protoplasm or chlorophyll as a stimulus, (2) the acid may affect the condition of solution of the bicarbonate or carbon dioxide in the solution so as to make it a more favourable source for assimilation, or (3) there may be a reserve of carbonic acid in the plant which the acid sets free

The last view is, of course, that of Adolph Mayer and Wilmott, and also of Nathansohn (1907) In support of this Benecke describes experiments in which the water used was boiled and distilled to free it from any carbon dioxide whatever Although on exposure to light there was no evolution of bubbles, on addition of dilute acid a good stream of bubbles resulted, followed by starch formation

Benecke, however, describes experiments which cannot be explained so easily In the case of *Potamogeton densus* exposed to light in water containing no carbon dioxide, he could obtain no indication of bubbling either with or without acid On the other hand, he obtained what he regarded as definite increase of assimilation by the addition of acid when he used either bicarbonate or carbon dioxide in the water In this case Benecke suggests that there may yet be a small reserve of carbonate which is too small to yield bubbles of oxygen on addition of acid, but which will give rise to an increase in the rate of bubbling if there is a substantial external source of carbon dioxide This is quite a conceivable explanation, as the oxygen released in absence of carbon dioxide and presence of acid might be so small that it could all dissolve in the water But Benecke suggests an alternative explanation, namely (2) above This depends on the relative quantities of CO_2, H_2CO_3 and the ions H and HCO'_3 into which carbonic acid is almost entirely dissociated Figures are quoted in support of this The addition of acid to a solution containing carbonic acid will depress the degree

of ionisation of the carbonic acid and increase the concentration of carbonic acid as a whole and the concentration of carbon dioxide If carbon dioxide enters the plasma as such, the increase in carbon dioxide concentration in the external solution resulting from the addition of acid would explain the increase in the rate of assimilation in *Potamogeton densus* under this condition

Wilmott and Benecke are in agreement that there is no evidence in support of the view of Angelstein (1911) to the effect that plants have the power " actively to split " bicarbonates

Recently Bose (1923, 1924) has recorded a considerable increase in the rate of oxygen evolution from water plants when nitric acid is present to the extent of one part in 2×10^9

Ammonium Salts —These appear to have a depressing effect on the rate of assimilation This was found to be the case by Ewart (1896) with ammonium carbonate, and Willstatter and Stoll (1918) make the statement that ammonium bicarbonate strongly depresses the rate of assimilation of leaves. A special study of the action of ammonium salts on photosynthesis has been made by Benecke (1921) He found that *Elodea* cultivated in solutions containing an ammonium salt is poorer in starch than similar plants cultivated in a similar solution without any source of nitrogen Some examples from among Benecke's experiments may be mentioned Two sets of destarched leaves of *Elodea* were exposed to sunlight on a July day, in solutions containing 1 per cent potassium bicarbonate In one case 0 5 per cent sodium sulphate was added to the solution, in the other case an equivalent quantity of ammonium sulphate After an hour, leaves in the former solution had developed starch in considerable quantity, but no starch was present in the leaves immersed in the solution containing the ammonium sulphate Similar results were obtained with chlorides Also in equivalent solutions of potassium bicarbonate and sodium bicarbonate starch was formed in 40 minutes, whereas none was formed in leaves immersed in solutions of ammonium bicarbonate of equivalent strength Even 0 01 per cent ammonium sulphate retards starch formation in *Elodea*, although it does not inhibit it

Various explanations of these results are possible Thus it might be that ammonium salts render the protoplast permeable to sugar, so that as this is produced in photosynthesis it diffuses out of the assimilating cells and the critical concentration at which sugar begins to be transformed into starch is never reached. In this connection Wachter's opinion (1905) that ammonium chloride brings about exosmosis from the cells of the bulb scales of onion may be mentioned However, Benecke could find no indication of such exosmosis

Another possibility is that the sugar formed in assimilation is at once synthesised along with the ammonium salt into complex organic nitrogen compounds Saposchnikoff (1894) thought he had shown that under prolonged treatment with nutrient salts and

subsequent exposure to damp weather assimilation proceeds without accumulation of carbohydrates, but with formation of proteins There is also the possibility that under the influence of ammonium salts a higher critical concentration of sugar is reached before starch formation commences. This, however, is negatived by the determination of the osmotic value of the cells (cf Stiles, 1924*b*), which is found to be not higher than, but either the same as or lower than, before the addition of ammonium salts to the external solution

The question appears to be in the main settled by Benecke's experiments with the bubbling method. Thus a shoot of *Elodea* exposed to the light of a 150-watt lamp at a distance of 30 cm in 100 c c. of a one per cent. potassium bicarbonate solution evolved 10 bubbles in 20 seconds After the addition of 0·1 gram of ammonium sulphate the rate of bubbling fell to 10 bubbles in 30 seconds, and after a short time had fallen to 10 bubbles in 90 seconds Addition of an equivalent quantity of potassium and sodium sulphates brought about no such reduction in the rate of bubbling. A number of other experiments yielded confirmatory results

There thus appears to be no doubt of the depressing action of ammonium salts on the rate of assimilation of *Elodea* In finding an explanation of this action Benecke refers to the rapidity with which ammonium salts enter plant cells (cf Birch-Hirschfeld, 1920, Stiles, 1924*c*) In this connection it is worth noting that the harmful effect of the ammonium salt can be antagonised by a calcium salt. Thus the depressing action of an ammonium salt is not observed when the latter is added to media containing calcium bicarbonate as source of carbon dioxide The action of a calcium salt in preventing or retarding the entrance of a salt of a monovalent kation is one of the best known facts of antagonism

Benecke, without any sufficient reason, in the opinion of the writer, considers that the harmful effect of ammonium salts is due to the rapid entrance of NH_3 or NH_4OH, not to NH_4 ions Whether ammonia enters more quickly than carbon dioxide and so depresses the concentration of the latter in the assimilating cell, or whether the ammonia acts harmfully in some other way, Benecke is unable to say

Sulphites—A depression in the rate of bubbling of *Elodea* in sunlight in presence of a small quantity of sulphite was observed by Kurt Noack (1920*a*) This is ascribed to a depressing action of the sulphite on the assimilatory process, but is not due to reaction of the sulphite with chlorophyll.

Hydrogen Cyanide—The action of hydrocyanic acid on photosynthesis, as investigated by Warburg (1919) in the case of *Chlorella*, appears to be different from the general action of acids, and is therefore considered separately here A checking of the rate of photosynthesis is observable in concentrations of the substance as

low as N/10,000, when the light intensity is high, whereas respiration is only similarly adversely affected in a concentration of N/100 On the other hand, when the light intensity is low, the action of the hydrogen cyanide is not nearly so marked Thus, if the light intensity is below that of the compensation point (see p 97), so that respiration exceeds photosynthesis, as, for example, with a light intensity of 440 lux, the rate of photosynthesis is found to be as high in a concentration of N/200 hydrogen cyanide as in absence of the substance altogether. In a light intensity of 19,000 lux, on the other hand, photosynthesis is completely suppressed

These observations, Warburg considers, throw considerable light on the mechanism of the assimilatory process, and his explanations of the observed results will therefore be discussed in a later chapter dealing with the mechanism of photosynthesis

Formaldehyde.—According to Bose (1923, 1924) one part in 10^9 of formaldehyde brings about an increase of 85 per cent in the rate of evolution of oxygen from the water plant *Hydrilla verticillata*

Various Toxic Substances.—Among substances other than those already noted above which have been found to depress assimilation may be mentioned turpentine vapour (Boussingault, 1868), quinine (Marcacci, 1895, Jacobi, 1899), strychnine (Weyl, 1881, Marcacci, *l c*) and morphine (Marcacci, *l.c.*), salicylic acid (Weyl, *l.c.*) and iodine (Jacobi, *l c*) Weyl found that one per cent solution of phenol inhibited photosynthesis, but that 0 25 per cent was insufficient to do so

Glycerol.—Experiments were made by Fromageot (1923*b*, 1924) on the effect of glycerol in different concentrations on the rate of photosynthesis of *Ulva lactuca* as measured by the rate of evolution of oxygen It was found that the rate of photosynthesis was reduced by presence of the glycerol, the reduction being greater the higher the concentration of the glycerol, evolution of oxygen stopping completely in a concentration of glycerol of 15 per cent. or higher After immersion in glycerol solutions of 10 per cent strength or lower for 15 minutes and then replacement in sea-water, complete recovery of photosynthetic activity was possible, but if the concentration of the glycerol had been between 10 and 35 per cent only partial recovery took place, while if the glycerol concentration had been above 35 per cent. no recovery took place The respiration was found to be affected differently by glycerol In 5 per cent. glycerol the rate of respiration was somewhat increased, but decreased progressively in higher concentrations

The author compares his results with those of Wurmser and Jacquot (1923) on the effect of temperature, and considers that they are due to alterations in viscosity They present another instance of the greater sensitiveness of the photosynthetic process

as compared with the respiratory mechanism to disturbing influences

Irrespirable Gases—Reference has already been made to the action of irrespirable gases on the rate of photosynthesis (see p 117) The depression of photosynthesis in flowering plants in an atmosphere of hydrogen, methane, nitrogen or carbon dioxide was observed by Boussingault (1868) by the eudiometric method, and his experiments were afterwards extended by Ewart (1896) with the bacterium method It was found that plants of *Chara* or *Elodea*, when kept in a stream of hydrogen either in light or darkness, lose their assimilatory power in the course of several hours, and even in a mixture of hydrogen and carbon dioxide photosynthetic activity is ultimately lost If the period of exposure is not too long the plants recover the power of assimilation In an atmosphere of pure carbon dioxide assimilation stops in one or two minutes

Mosses were found to be very resistant to the depressing effect of immersion in an atmosphere of hydrogen, as well as to the poisonous action of an atmosphere of carbon dioxide, and in *Bryum cæspitosum* immersion in an atmosphere of hydrogen for a week in darkness was found to produce little ill effect In *Orthotrichum affine* and *Dicranum scoparium*, at any rate, it appears that depression in the rate of photosynthesis ultimately takes place, but here, again, recovery may take place on re-transference to a normal atmosphere.

Wounding

In order to examine whether wounding had a stimulating effect on photosynthesis, Kostytschew (1921*b*) compared the rates of photosynthesis in unwounded leaves of *Betula pubescens* and *Lamium album* with those in leaves of the same two species which had been wounded with a series of strokes of a fine-pointed glass needle In five out of six experiments the rate of photosynthesis in the wounded leaf was somewhat less per hour per square decimetre than in the unwounded leaf, and in the sixth case the difference between the rates of assimilation in the two leaves was very little There is thus no evidence that wounding stimulates photosynthesis

As the rate of photosynthesis is still considerable, Kostytchew concludes that the general cytoplasm plays no part in the binding and reduction of carbon dioxide, but that this process takes place exclusively in the chloroplasts, and he agrees with the opinion of Engelmann (1881) and Ewart (1896, 1897*c*, 1898*a*) that isolated chloroplasts can assimilate (But see also Kny (1898) on this point)

Kostytchew appears to have taken no account of respiration and of the influence of wounding on this process For this reason it appears that his results leave the question still in doubt.

Electrical Conditions

The effect of the passage of an electric current through a leaf on the rate of photosynthesis has been examined by Thouvenin (1896) in the case of water plants (*Elodea canadensis*, *Myriophyllum spicatum* and *Potamogeton perfoliatus*) and by Pollacci (1905, 1907*a*) in the case of a number of land and water plants, but particularly *Calla æthiopica* and *Arum italicum* Thouvenin found that when a direct current of 0 0027 ampere passed through a shoot of *Elodea* from the apex to the base the rate of bubbling was 52 bubbles in 4 minutes, whereas when no current was passing the rate was only 29 bubbles in the same time A number of other results similar to this were obtained. Pollacci determined the rate of photosynthesis in electrified and non-electrified leaves by the dry-weight method and the saccharification method, and also found an appreciably higher rate of assimilation in the electrified leaves as compared with the non-electrified ones when the current was weak, one of a few microamperes, and flowed from the base to the top of the lamina. When the current flowed in the reverse direction assimilation appeared to be retarded The more favourable action of the current when it flows from the base to the apex has been confirmed by Koltonski (1908)

These results are very suggestive, but they are open to criticism on account of the errors to which the determination of the assimilation is liable by the methods employed If they should be confirmed by more exact methods of determining photosynthesis, it still would seem improbable that Pollacci's opinion to the effect that the electric current has supplied energy for the photosynthetic process can be the whole explanation, for the energy supplied by the very weak currents used is negligible in comparison with the energy supplied normally by light

It is possible that the electrical condition of the atmosphere may have some influence, direct or indirect, on photosynthesis Henrici (1921*b*) states that certain species from Alpine habitats showed increased photosynthetic activity when placed in an atmosphere ionised by means of thorium oxide Individuals of the same species from a lowland habitat only exhibited this behaviour when exposed to a low intensity of illumination , otherwise the effect of ionisation of the air was the reverse Temperature was found not to affect these observed results, but the effect of ionisation was found to decrease progressively with increase of carbon dioxide concentration The suggestion is made that the effect on photosynthesis may be brought about by increase in the rate of diffusion of the carbon dioxide

The information available on the influence of radium radiations on photosynthesis appears to be quite indefinite (see Hébert and Kling, 1909).

Chlorophyll Content

The work of F F Blackman on the influence of various essential environmental factors made it clear how each one of these factors (carbon dioxide concentration, light intensity and temperature) is related to the rate of photosynthesis From this work it is evident that the relations of these factors to the rate of photosynthesis cannot be expressed in definite physical and chemical terms, but that the experimental results obtained can be explained on the principle of limiting factors. Subsequent work on the external factors has not affected the validity of the general principle, although it is now a question whether the condition in which one factor is limiting passes, with increase in the value of this factor, abruptly or gradually into the condition where some other factor is limiting

The next step in a consideration of the influence of conditions on photosynthesis is naturally an inquiry into the relation between the value of internal factors and rate of photosynthesis The most obvious of the internal factors is the green pigment chlorophyll, and naturally our first inquiry will be whether the quantity of chlorophyll present in the assimilating cell can act as a limiting factor in the same way as the external conditions.

The question was rendered difficult before Willstätter and Stoll had investigated the leaf pigments, because there was no reliable method of estimating the quantity of chlorophyll and yellow pigments in assimilating organs The working out of such methods by Willstätter and Stoll (cf. Chapter III) rendered a successful attack on the problem possible

That the rate of photosynthesis might be dependent on the amount of chlorophyll had, of course, been recognised earlier Thus, C. Weber (1879) found that equal areas of the leaves of different species formed different quantities of dry matter in the same time and under the same conditions Haberlandt (1882) determined the number of chloroplasts per unit area in the plants used by Weber, and found a very remarkable parallelism between the number of chloroplasts and the assimilatory activity, as the following table shows But it does not follow, of course, that all

TABLE 24

Relation between Photosynthetic Activity and Number of Chloroplasts

(Data from Weber and Haberlandt)

Species	Photosynthetic activity per unit area. (*Tropæolum* put equal to 100)	Number of chloroplasts per unit area (*Tropæolum* put equal to 100)
Tropæolum majus	100 0	100
Phaseolus multiflorus	72 0	64
Ricinus communis	118 5	120
Helianthus annuus	124 5	122

chloroplasts contain the same amount of chlorophyll, and Haberlandt himself realised later (1909, 1914) that probably the assimilatory activities of the chloroplasts of different species exhibit specific differences

Our knowledge of the influence of chlorophyll content on the rate of photosynthesis rests almost entirely on the researches of Willstatter and Stoll (1915*a*, *b*, *c*, 1918), who determined the rate of photosynthesis of a considerable number of leaves, the chlorophyll content of which they estimated For determining the rate of photosynthesis they employed the continuous gas current method (see Chapter V), a stream of gas containing 5 per cent by volume of carbon dioxide passing over the leaves at a rate of 3 or 4 5 litres per hour A high intensity of illumination was employed, usually 48,000 lux,[1] although intensities up to 130,000 lux could be employed The temperature usually employed was 25°, although some experiments were carried out at 30° C. These conditions are such that the rate of photosynthesis of a normal leaf cannot be increased by increasing the carbon dioxide concentration or the light intensity, that is, neither light nor carbon dioxide is a limiting factor The rate of photosynthesis must therefore depend, presumably, on the temperature and on internal factors

The relation between the rate of photosynthesis and chlorophyll content was expressed by Willstatter and Stoll as the quantity of carbon dioxide absorbed in one hour per unit of chlorophyll Both the carbon dioxide and chlorophyll are measured in the same units (for example, in grams) This quantity is called the " assimilation number," which is therefore

$$\frac{\text{assimilation of carbon dioxide in grams per hour}}{\text{chlorophyll content in grams}}$$

If the rate of assimilation depends only on the content of chlorophyll, the assimilation number should be constant, if, on the other hand, factors other than chlorophyll content influence the rate of photosynthesis, the assimilation numbers will show variations, as we should no longer expect proportionality between chlorophyll content and rate of photosynthesis, while if chlorophyll content acts in the same way as an environmental factor and is in excess, while an external factor is limiting the rate, we should expect alteration in the chlorophyll content to produce no alteration in the rate of assimilation

Willstatter and Stoll compared the assimilation numbers of the fully developed leaves of a number of different species, of leaves in different stages of development and at different seasons of the year, of green and yellow varieties of the same species, of etiolated leaves becoming green, of chlorotic leaves, of leaves completely

[1] The lux is the intensity of illumination of a surface 1 metre distant from 1 Hefner candle, the illuminated surface being in a plane at right angles to the line joining the surface and the source of light.

devoid of chlorophyll, of leaves of green and anthocyan-containing varieties of the same species, and of the pericarp of fruits Their researches thus cover a remarkably wide field The chief results of these researches are summarised below

Normal Leaves—The assimilation numbers of fully developed leaves of a number of different species are shown in Table 25. In

TABLE 25

ASSIMILATION NUMBERS OF ORDINARY LEAVES

(Data from Willstätter and Stoll)

Species	Chlorophyll content of 10 g fresh leaves (in mg)	CO_2 absorbed per hour by 10 g. fresh leaves (in g)	CO_2 absorbed per hour by 1 sq dec. leaf surface.	Assimilation number.
Æsculus Hippocastanum	24 7	0 159	0 033	6 4
Acer Negundo	24 8	0 192	0 040	7 7
Acer Pseudoplatanus	40 0	0 207	0 027	5 2
Ampelopsis quinquefolia	28 8	0 178	0 028	6 2
Tilia cordata	28 1	0 188	0 028	6 6
Laurus nobilis	12 7	0 075	0 019	5 9
Sambucus nigra	22 2	0 146	0 034	6 6

selecting the leaves care was taken to avoid both young and old leaves as well as those showing poverty in chlorophyll These values were all obtained with a carbon dioxide concentration of 5 per cent by volume, a light intensity of 48,000 lux, and at a temperature of 25° C The assimilation numbers are approximately constant, varying between 5 2 and 7 7 Somewhat higher values were obtained at 30° C. with plants from the hot-house, the assimilation numbers varying from 6 5 with *Hydrangea opuloides* to 9 1 with a species of *Primula*

The results given in Table 25 suggest that the rate of photosynthesis in different species might be proportional to the content of chlorophyll when neither carbon dioxide concentration nor light intensity is a limiting factor There are, however, other plants which, under the same conditions of experimentation, give much higher assimilation numbers. Thus leaves of *Helianthus annuus* gave, in three different experiments, assimilation numbers of 14 0, 16·7 and 10·9 *Cucurbita Pepo* and *Clerodendron trichotomum* were found to give assimilation numbers of 12 1 and 12 3 respectively, while the hot-house plant *Pelargonium peltatum* at 30° C. yielded an assimilation number of 14 5 It is clear, therefore, that in the fully developed leaves of ordinary mesophytic plants the rate of photosynthesis, under the conditions of experiment, is not proportional to the quantity of chlorophyll present This fact becomes more obvious when the assimilation of leaves of the same species but in different stages of development is examined

Leaves in Different Stages of Development—The assimilation numbers of leaves of the same species at different times in spring were found to vary with the age of the leaf As the leaf gets

older the chlorophyll content increases, and along with this the rate of photosynthesis also increases, but not at the same rate Hence with increase in chlorophyll content the assimilation number falls In the earlier stages of development, however, there is sometimes observed an increase in the assimilation number before its fall. Examples from among the results of Willstatter and Stoll are given in Table 26 The values were again found with 5 per cent. carbon dioxide concentration, a light intensity of 48,000 lux and a temperature of 25° C Younger and older leaves of the same plant gathered at the same time also showed higher assimilation numbers in the case of the former. Thus young green leaves of the lime (*Tilia cordata*) were found to possess an assimilation number of 14 2, while lower dark green leaves from the same shoot gave an assimilation number of 6 6.

TABLE 26

ASSIMILATION NUMBERS OF LEAVES OF THE SAME SPECIES IN DIFFERENT STAGES OF DEVELOPMENT

(Data from Willstätter and Stoll)

Species.	Date.	Chlorophyll content of 10 g fresh leaves (in mg)	CO_2 absorbed per hour by 10 g fresh leaves (in g)	CO_2 absorbed per hour by 1 sq dec leaf surface (in g)	Assimilation number.
Sambucus nigra	1 May	11 7	0 143	0 046	12 2
,, ,,	8 ,,	23 1	0 227	0 057	9 8
,, ,,	14 July	23 5	0 145	0 032	6 2
Tilia cordata	4 May	8 3	0 088	0 015	10 6
,, ,,	12 ,,	11 5	0 183	0 024	16 0
,, ,,	5 June	28 8	0 205	0 029	7'1
,, ,,	25 ,,	28 1	0 185	0 028	6 6
Quercus Robur	11 May	6 6	0 072	0 013	10 9
,, ,,	20 ,,	8 6	0 136	0 024	15 8
,, ,,	9 June	21 6	0 194	0 038	9 0
,, ,,	20 ,,	25 0	0 196	0 041	7 8

Autumn Leaves—In autumn leaves the relation between chlorophyll content and rate of photosynthesis varies greatly from species to species Thus, some leaves, such as those of *Sambucus nigra*, which retain their green colour until they fall, retain approximately the same assimilation number until the end; the rate of photosynthesis thus decreases proportionately to the decrease in chlorophyll content In other cases the assimilation number decreases with age, although the leaves remain deep green until they fall In some cases where the leaves become yellow with decreasing chlorophyll content, the assimilation number first rises and then falls again In other cases the assimilation numbers of green and yellowed leaves are approximately the same, while in yet others there is a very considerable fall in the assimilation number as the chlorophyll disappears

Leaves Poor in Chlorophyll (*Yellow Varieties*)—An attempt was made by Plester (1912) to compare the rates of photosynthesis

in green and yellow varieties of a number of species by the use of the half-leaf method Apart from the fact that he found the rate of photosynthesis always much less in leaves of low chlorophyll content, he was not able to make any generalisations with regard to the relation between chlorophyll content and rate of photosynthesis, nor is this to be wondered at having regard to the lack of accuracy attaching to the method of assimilation measurement employed

The question was very thoroughly examined by Willstatter and Stoll It was found that the leaves of yellow varieties exhibit very much higher assimilation numbers than those of the normal green forms of the same species Thus the assimilation number of the leaves of a yellow variety of *Quercus Robur* was found to be 55 as compared with 7 8 found for the leaves of the ordinary green form. The mean of two determinations of the assimilation number of the leaves of the normal form of *Sambucus* was found to be 6 4, whereas the mean of three determinations of the assimilation number of leaves of the yellow variety was 113 The determinations were made, as before, with a carbon dioxide concentration of 5 per cent, a light intensity of 48,000 lux and at a temperature of 25° C Similar results were obtained with leaves of the elm at 25° C and with leaves of *Sambucus nigra*, *Acer Negundo* and *Quercus Robur* at 30° C At the higher temperature, however, the difference between the assimilation numbers of green and yellow leaves is reduced, and the same temperature effect was found with the leaves of green and yellow varieties of elm, the results for which at temperatures of 15° and 25° C are shown in the following table The

TABLE 27

ASSIMILATION NUMBERS OF LEAVES OF GREEN AND YELLOW VARIETIES OF *Ulmus*

(Data from Willstätter and Stoll)

Temperature in Centigrade degrees	Variety	Chlorophyll content (in mg).	CO_2 absorbed (in g)	CO_2 absorbed per sq metre (in g)	Assimilation number
15	Green	13 0	0 059	1 4	4 5
,,	Yellow	0 95	0 056	1 7	59 0
25	Green	13 0	0 089	2 1	6 9
,,	Yellow	0 95	0 075	2 3	79·0

carbon dioxide concentration was 5 per cent as before, the light intensity 24,000 lux and the weight of fresh leaf used in both cases 8 0 grams

Etiolated Leaves becoming Green.—The examination of the photosynthetic activity of etiolated leaves while they become green is of particular interest, because during this process the quantity of chlorophyll is continually increasing Consequently, if chlorophyll content acts as a simple limiting factor it is to be

expected that during greening the rate of photosynthesis will be approximately proportional to the chlorophyll content until some other factor is limiting

The earliest work on this subject appears to be that of Wiesner (1877), who found that during the early stages of greening of barley seedlings less carbon dioxide is evolved from the plants during illumination than in the dark, from which he concluded that carbon dioxide is concerned in the development of chlorophyll from "etiolin." Ewart (1897*a*) was unable to discover any simple relation between chlorophyll content and the rate of photosynthesis of etiolated plants, although he concluded from his observations by means of the bacterium method that some etiolated cells that are neither too old nor too young can assimilate, from which it has been supposed that "etiolin" is an assimilatory pigment [1]

More recently the subject was examined by Miss Irving (1910), working in Blackman's laboratory. Etiolated shoots of barley and broad bean (*Vicia Faba*) were intermittently exposed to light and darkness, and while the shoots were becoming green the rate of photosynthesis was measured during the periods of illumination by determining the depression in the rate of evolution of carbon dioxide as compared with that respired in the dark Miss Irving found that not only had etiolated shoots no power of photosynthesis, but that shoots that had developed a considerable green colour exhibited no photosynthetic activity It was concluded that the amount of chlorophyll present is never a factor limiting the rate of photosynthesis during the early stages in the development of the assimilating organs, and that some other component part of the photosynthetic machinery, which is not developed by illumination so rapidly as the green pigment, controls the beginning of complete assimilatory activity

Willstatter and Stoll's results with etiolated leaves of *Phaseolus vulgaris* and *Zea Mays* gave different results In these cases the rate of photosynthesis increased regularly with the development of chlorophyll in the leaves In fact, in the first stages, when chlorophyll content is very low, the assimilation numbers are very high and of about the same order as those found for the leaves of yellow varieties The increase in the rate of photosynthesis with increase in chlorophyll content in the case of *Phaseolus* is shown in Table 28, which also shows how the assimilation number decreases as the green pigment develops It is difficult to find an explanation of the different results of Miss Irving and of Willstatter and Stoll

[1] On this point very diverse opinions are held Thus Pfeffer (1900) says, "Ewart has, however, conclusively proved that in the absence of all traces of chlorophyll, etiolated chloroplasts may show a faint power of carbon dioxide assimilation" On the other hand, Jost (1908) remarks, "At present we may be permitted to ignore Ewart's statements" Also, more recent work (Willstätter and Stoll, 1913, Coward, 1924*b*) has shown that "etiolin" consists of the usual carotinoids of leaves, that is, xanthophyll and carotin, so that photosynthesis is not to be expected in etiolated plants

It is to be observed that whereas Miss Irving used a low carbon dioxide concentration, that of the plants' own respiratory carbon dioxide, Willstatter and Stoll used a high concentration (5 per cent) of this gas Willstatter and Stoll also used a high light intensity (48,000 lux), whereas Miss Irving used either the light from a north window or from a pair of Keith high-pressure incandescent gas-burners It might seem at least possible that in Miss Irving's experiments either light or, more probably, carbon dioxide concentration, limited the rate of photosynthesis However, under conditions of experiment similar to those of Miss Irving it was found by Willstatter and Stoll that leaves which had developed only 3 to 6 per cent. of their normal chlorophyll content were able to use completely in assimilation their respiratory carbon dioxide.

TABLE 28

ASSIMILATION NUMBERS OF ETIOLATED LEAVES OF *Phaseolus vulgaris* BECOMING GREEN

(Data from Willstätter and Stoll)

Duration of Illumination.	Appearance	Chlorophyll content of 10 g fresh leaves (in mg)	CO_2 absorbed by 10 g. fresh leaves (in g)	CO_2 absorbed by 1 sq dec leaf surface (in g).	Assimilation number
Not previously illuminated .	Pure yellow	0 2	0 014	—	70
6 hours	Greenish-yellow	0 7	0 091	—	133
2 days.	Yellow-green	8 0	0 192	0 044	24
4 days	Grass-green	15 6	0 208	0 040	13 3
Control grown in light .		18 6	0 174	0 030	9 4

An explanation of the divergence in results comes from the work of Briggs (1920) carried out in Blackman's laboratory Working with *Phaseolus vulgaris* principally, but also with young buds of *Vicia Faba* and young leaves of oat (*Avena sativa*), Briggs found that the assimilatory power of the young leaves depends on their age rather than on the content of chlorophyll. Light was obtained from a 32 candle-power filament lamp, and a concentration of carbon dioxide of 5 per cent was used The rate of photosynthesis was measured by Blackman's palladium black method (see Chapter V), which, by allowing the use in the assimilation chamber of so low a pressure of oxygen that further development of chlorophyll is prevented, allows the maintenance of constant chlorophyll content By bringing plants from the dark and exposing them to illumination at different times, it was found that the rate of assimilation was determined by the number of days that had elapsed from sowing, not by the content of chlorophyll Thus, plants which had been exposed to light for 25 hours showed no assimilatory power on the ninth day after sowing, while plants from seed sown at the same time which had only been exposed to light for 13 hours but which were four days older, showed a very appreciable rate of photosynthesis

Now it appears that Miss Irving's material had been taken from plants only 5 or 6 days old, whereas the leaves used by Willstatter and Stoll were from plants 14 or 15 days old. It may be expected, therefore, that in the plants used by Willstatter and Stoll the assimilatory apparatus had developed with the exception of the chlorophyll, so that as the amount of the latter increased it is to be expected that the rate of photosynthesis would increase also In Miss Irving's experiments, on the other hand, the rest of the assimilatory apparatus was not developed in such young plants, so that increase in chlorophyll content was not able to bring about photosynthetic activity.

Briggs's results thus lead to the conclusion that " the photosynthetic potentiality of this factor rapidly increases with age, day by day, whether the leaf is in the light or in the darkness, and even though there is no concurrent increase in the amount of chlorophyll "

In a subsequent extension of this work, Briggs (1922*a*) found that seedlings fall into two classes with regard to the development of photosynthetic activity In *Phaseolus*, *Ricinus* and *Zea*, where a specialised photosynthetic organ is developed different from the storage organ, photosynthetic activity, as we have seen, is not developed until some time after germination, whereas in *Helianthus*, *Acer* and *Cucurbita*, in which the cotyledons are storage organs and subsequently become the first assimilating organs of the seedling, the photosynthetic activity is fully developed at germination, and, in consequence, there is no lag between the development of chlorophyll and the development of full photosynthetic activity

Chlorotic Leaves—In spite of the low chlorophyll content of chlorotic leaves from plants of *Helianthus annuus* and *Zea Mays* grown in water culture without iron, the assimilation numbers of such leaves were found by Willstatter and Stoll to be about the same, or even lower, than those of normal leaves When this result is compared with those given by the leaves of yellow varieties it is clear that the chlorophyll, in spite of its small amount, is only partially utilised in chlorotic leaves

Leaves containing Anthocyanin—Leaves of the red variety of *Acer Pseudoplatanus* were found to give approximately the same rate of assimilation and the same assimilation number as the normal green form The content of anthocyanin is thus without direct influence on the assimilatory activity

This result is in harmony with the conclusion of Griffon (1899*a*), to the effect that where varieties containing anthocyanin possess leaves of the same thickness and chlorophyll content as those of the green form, the rates of photosynthesis are the same in the two kinds of leaves In some cases the photosynthetic activity of the anthocyanin-containing variety was found by Griffon to be less than that of the green form, but in such cases the leaves were

found always to be thinner, or poorer in chlorophyll, than the leaves without anthocyanin

Leaves without Chlorophyll—Experiments made by Willstatter and Stoll with albino varieties indicate that in complete absence of chlorophyll photosynthesis also fails

Pericarps—The assimilation numbers found by Willstatter and Stoll for the pericarp of ripening pears were normal, varying from 6 1 to 11 1 In the case of the grape, however, very low values (3 3 to 0) were found

The Protoplasmic Factor

We have seen from the consideration of the relation of chlorophyll content to the rate of photosynthesis that the facts observed can only be explained by supposing that some internal factor, besides chlorophyll, is necessary for photosynthetic activity That there must be this essential internal factor in photosynthesis has been realised for a long time Thus Ewart (1896, 1897*a*) concluded, from his studies on the inhibition of assimilation by different means, that chlorophyll is not the only internal factor He says, for example, "A careful study of the above results enforces the conclusion that, although the presence of a certain amount of chlorophyll is necessary before any evolution of oxygen can take place, nevertheless, in determining the development of the power of assimilation and the stage at which an evolution of oxygen is possible, equally important factors, of probably plasmatic origin, also enter into play" Again, Pfeffer (1897, 1900) says, "It is evident that the mere presence of chlorophyll in the cytoplasm will not necessarily confer a power of assimilating carbon dioxide upon it, for the process can only proceed when the proper functional relationship exists between the two."

Engelmann (1888*b*) expressed the opinion that it is the colourless stroma that is the importantly active constituent of the assimilating cell, and that the chlorophyll is only a sensitiser Willstatter and Stoll (1918), as a result of their work on the relation of chlorophyll content to photosynthesis, in which they clearly show that with equal external conditions the rate of photosynthesis is not necessarily proportional to chlorophyll content even in the same species, have produced the clearest evidence of the existence of this internal factor They conclude, largely from experiments on the influence of light intensity and temperature on the rate of assimilation of green and yellow varieties respectively, that the internal factor is an enzyme They find that photosynthesis in leaves rich in chlorophyll is more influenced by temperature than in yellow leaves of the same variety, while with varying light intensity photosynthesis in yellow leaves is more affected than in green leaves In leaves of high chlorophyll content the other internal factor is limiting, or in relative minimum, so that this chiefly governs the rate of photo-

synthesis Hence the chlorophyll cannot be completely utilised, and so much lower assimilation numbers are obtained than in chlorophyll-poor leaves Increase in temperature in this case brings about an increase in the rate of photosynthesis, because the part of the whole process controlled by the enzyme is speeded up by the increase in temperature in the way characteristic of enzyme actions In the leaves poor in chlorophyll, however, the chlorophyll is less developed than the enzyme, and in consequence the chlorophyll is much more utilised than in the case of the green varieties, so that much higher assimilation numbers are obtained Increase in temperature will not have so much effect on the rate of photosynthesis in these leaves, because the part of the whole process in which chlorophyll is concerned will limit the rate of the whole process, and this, being dependent on light, is an action with low temperature coefficient characteristic of photochemical reactions. Increase in light intensity, on the other hand, will effect a considerable increase in the rate of photosynthesis, as it will increase the rate of the photochemical stage of the photosynthetic process in which the chlorophyll is involved It will be observed that these considerations of Willstatter and Stoll on the protoplasmic factor in photosynthesis involve the postulation of two distinct stages in the photosynthetic process, a photochemical reaction and an enzyme action A further consideration of this will be deferred to a later chapter, where the stages in the photosynthetic process will be dealt with

Spoehr and McGee (1923) find that if the supply of available carbohydrates in the leaf is reduced by starvation, there is a progressive decrease in both the rate of respiration and rate of photosynthesis Similarly, if leaves which have been depleted of their carbohydrates by a period in the dark are exposed to light, both the rates of respiration and of photosynthesis increase with time as the carbohydrate content increases Spoehr and McGee appear to see in this connection of photosynthesis and respiration the possibility that the protoplasmic factor in photosynthesis is connected with respiration, and the necessity of oxygen for photosynthesis can be adduced in support of this view This work is still in a preliminary stage at the time of writing, and it will be well to wait for further data before entering into a discussion of the meaning of the relation between the two processes

The case of the saprophyte *Neottia nidus-avis* deserves mention in connection with the protoplasmic factor in photosynthesis This saprophyte is generally regarded as containing a little chlorophyll, the presence of which is masked by other pigment (Wiesner, 1872; Molisch, 1905), although some doubt has been expressed on the point (Schimper, 1885*a*, Lindt, 1885) Drude (1873) concluded that the slight amount of chlorophyll present is yet sufficient to bring about a feeble evolution of oxygen in strong illumination, although Prillieux (1874*a*) could obtain no evidence of this,

while Pfeffer (1900) makes the statement that "the saprophyte *Neottia* and the parasites *Orobanche* and *Cuscuta* contain a little chlorophyll[1] and are able to produce a small and perhaps unnecessary portion of their organic food by photosynthesis." (See Ewart, 1896.) Bonnier and Mangin (1884), however, were unable to find the slightest trace of carbon dioxide absorption by *Neottia*. This has been confirmed by Willstatter and Stoll, who conclude that in this plant, although chlorophyll is present, the enzymic system, that is, the protoplasmic factor, is incompletely developed, and hence photosynthesis cannot occur. F. Weber (1920) also failed to obtain any evidence of the evolution of oxygen by *Neottia* by the bacterium method, although he obtained evidence of it by the indigo-carmine method. He considers that the question of photosynthesis in this plant has not yet been conclusively settled. He points out that, according to Wilschke (1914), the chlorophyll in this plant is exclusively chlorophyll *a*, but, in view of the poverty of the brown algæ in regard to chlorophyll *b*, it is difficult to suppose that the absence of appreciable photosynthetic activity in *Neottia* can be attributed simply to the absence of this chlorophyll component.

In another purple saprophytic orchid, *Limodorum abortivum*, in which apparently more chlorophyll is present than in *Neottia*, a measurable amount of photosynthesis, although insufficient for the needs of the plant and not equal to the respiration, is reported to take place (Chatin, 1874). Also, the observation of Bonnier (1891) that no assimilation could be detected, even in strong light, in the green root parasites *Euphrasia spp.*, and some species of *Rhinanthus* and *Bartsia*, was not confirmed by Ewart (1896), who found, by means of the bacterium method, active assimilation in species of all three genera. This result has been confirmed by Heinricher (1910, 1917, 1924), and by Kostytschew, Tswetkowa and Tillmann (1924), who have found that in the Rhinanthaceæ photosynthesis is as vigorous as in autotrophic plants. The insectivorous plants *Drosera rotundifolia* and *Pinguicula vulgaris* are also, according to Kostytschew (1923), quite normal in their photosynthetic activity.

It has been observed that plants in a pathological condition have a lower photosynthetic activity than plants of the same species in a healthy condition. Miss Long (1919) found that plants of *Avena* attacked by *Puccinia* possessed a photosynthetic activity only 48 per cent. of that of healthy individuals. A similar reduction in the rate of photosynthesis was observed in the case of *Phaseolus* attacked by the red spider *Tetranychus*. The lowered rate of photosynthesis in these cases may very possibly be due to imperfection in the protoplasmic factor rather than to a reduction in chlorophyll content, but we are without information which enables this point to be settled.

[1] See Temme (1883) for the case of *Cuscuta europæa*.

Anatomical Structure

Reference has been made in the section of this chapter dealing with the influence of light intensity on photosynthesis to the different behaviour of the leaves of sun and shade plants. It is well known that in general the leaves of sun and shade plants show considerable difference in anatomical structure, and it is interesting to inquire whether any difference in photosynthetic activity is correlated with difference in anatomy

Among the Umbelliferæ, Géneau de Lamarlière (1893) found that leaves with two or three layers of palisade, such as those of *Seseli* and *Fœniculum*, assimilate two or three times as fast as those with only one layer of palisade, such as the leaves of *Angelica sylvestris* and *Heracleum* This is completely understandable, as the thicker leaves will, per unit of leaf surface, possess a higher content of chlorophyll, while the protoplasmic factor, it is to be supposed, will also have a higher value Similarly, Griffon (1899*a*) found that leaves of *Ligustrum ovalifolium* of a paler green tint evolved oxygen more rapidly in assimilation than leaves of the same species of a deeper green, a difference which could be correlated with the fact that the paler green leaves had a mesophyll 324μ thick, of which 175μ was accounted for by palisade, while the deeper green leaves were only 243μ thick with a palisade 116μ thick. On the other hand, the pale green leaves of *Spiræa Billardi* with one layer of palisade 46μ deep assimilated at practically the same rate as the deeper green leaves of *Spiræa Revesiana* with four layers of palisade having a thickness of 108μ Again, two varieties of *Canna*, one with paler thinner leaves and the other with thicker leaves of a deeper green, showed no differences in photosynthetic activity. In such cases it is possible that some external factor was limiting in the experiments, or there may be a difference in the development of the protoplasmic factor in the different cases Or it may be that in the thicker leaves the outer layers of cells absorb all the energy utilisable for photosynthesis, although the experiments of Griffon (1899*b*) and of Ursprung (1917), in which, contrary to the observations of Nagamatz (1887), assimilation was found to take place in a leaf screened by another leaf, render this explanation very doubtful Again, other anatomical characters apart from thickness may, by influencing the rate of entrance of carbon dioxide into the assimilating cells, or the absorbing surface in the cells, or the general relations of the different parts of the assimilatory apparatus, affect the rate of photosynthesis To quote from an undated work of Griffon (about 1900), "Le développement du tissu palissadique et des lacunes, l'importance relative des tissus chlorophylliens et des tissus incolores dans un organe, et leur mode de répartition, la distribution des chloroleucites dans les cellules, le rôle d'écran joué par les assises vertes vis-à-vis des assises situées plus profondément, peut-être l'épaisseur de la cuticule, la présence

des cires épidermiques, le nombre des stomates et le développement des poils, tels sont donc les facteurs anatomiques que l'on peut invoquer pour expliquer les variations de l'énergie assimilatrice"

Géneau de Lamarlière (1892) found that leaves which have developed in the sun assimilate more rapidly than those which have developed in the shade, and Lundegårdh's recent work is in harmony with this conclusion.

The different relation between light intensity and rate of photosynthesis found by Lundegårdh in sun and shade plants (see p. 87) was explained by him (1922*b*) as due to internal structure If s is the area of the surface of the intercellular spaces, and m the mass of the chloroplasts, s/m is five times as great in sun plants as in shade plants. In the latter the small ratio of the cell absorbing surface to the mass of the chloroplasts is supposed to act as an internal limiting factor, limiting the rate at which the carbon dioxide can diffuse into the cells and so to the chloroplasts Hence, with progressive increase in the value of the external conditions, the rate of assimilation cannot reach so high a value in shade leaves as in sun leaves, owing to the operation of this internal limiting factor

The observation of Kostytschew (1921*c*) that during the summer night of subarctic regions an absorption of carbon dioxide could be observed in *Pinus Strobus* and *Abies sibirica*, but not in any Angiosperms examined, is probably to be related to anatomical structure It is possible that a closure of stomata takes place in darkness in the latter plants but not in the Coniferæ mentioned

Accumulation of Products

It is to be expected that if photosynthesis continues while removal of the products of the process from the assimilating cells is prevented, a time will come when no further photosynthesis is possible owing to the accumulation of the products of the reaction. Evidence of this was obtained by Saposchnikoff (1889, 1890*a*, 1891*b*), who experimented with detached leaves of *Vitis* and *Rubus* He found, for example, that leaves of *Vitis Labrusca* cease to form any more carbohydrate when the content of the latter reaches 17 to 25 per cent of the dry weight of the leaf The same worker further showed (1893) that isolated leaves of *Vitis vinifera* cease to produce any more carbohydrate after exposure to light for 6 or 7 days when they contain carbohydrates to the extent of 23 to 29 per cent of the dry weight of the leaf, the result being much the same whether the leaf-stalks are kept in distilled water or in a nutrient solution If, however, the leaves were kept in an atmosphere with a high percentage of carbon dioxide (one with 20 per cent of this gas at the commencement of the experiment), the maximum amount of carbohydrate reached 30 to 35 per cent of the dry weight, and this after only three days, a result attributed

to the more rapid rate of photosynthesis in the high concentration of carbon dioxide and to the leaves retaining their normal condition more nearly during the shorter period By the use of the bacterium method these observations were confirmed and extended to a number of plants of different groups by Ewart (1896) The plants used included ordinary starch formers among the higher plants (*Rubus odoratus, Vitis vinifera, V Lubrusca* and *Æsculus Hippocastaneum*), *Allium Cepa*, which forms sugar but not starch, water plants (*Elodea canadensis* and *Utricularia vulgaris*), mosses (*Dicranum scoparium, Catherinea undulata, Bryum cæspititium* and *Mnium stellare*) and an alga (*Œdogonium*) The same author also showed that the conclusion of Dehnecke (1880) that chloroplasts that store starch act merely as leucoplasts and have no assimilatory function was erroneous Such chloroplasts, in *Pellionia Daveauana*, for example, cease to assimilate owing to the accumulation of carbohydrate within them , on removal of the starch photosynthesis takes place in the parts of the plant containing these chloroplasts.

A Muller (1904) also came to the conclusion that accumulation of products of photosynthesis retards the rate of this process. He found that plants which store their carbohydrates as sugar produce less material in their leaves during assimilation than plants which temporarily store starch in their leaves, a result which can be explained on the view that solid starch is removed from the reaction medium while the soluble sugars are not

On the Actual Rates of Photosynthesis

The actual rate of photosynthesis of an assimilating organ thus depends on a number of internal and external factors, although at any particular moment only one, or a very few, of these factors may actually determine the rate The lower limit of the rate of photosynthesis is clearly zero when any one of the essential conditions is lacking The highest recorded rate of photosynthesis is that determined by Willstatter and Stoll (1918) for leaves of the sunflower (*Helianthus annuus*) exposed to an atmosphere containing 5 per cent (by volume) of carbon dioxide and to a light intensity of 48,000 lux at a temperature of 25° C This rate of photosynthesis was 80 mg of carbon dioxide per sq decimetre per hour (8 0 grams per sq metre per hour) With *Cucurbita Pepo* under similar conditions the highest rate found by the same workers was 63 mg. of carbon dioxide per sq decimetre per hour Blackman and Matthaei (1905) found that leaves of *Helianthus tuberosus* at a temperature of about 30° C exposed to strong sunshine in August in an atmosphere containing 6 3 per cent (by volume) of carbon dioxide assimilated 58 mg of carbon dioxide per hour per sq decimetre But these species appear to be among those capable of rather exceptionally high rates of photosynthesis With most species under similar conditions decidedly lower values were found

Thus leaves of the plane (*Acer Pseudoplatanus*) assimilated only 27 mg. of carbon dioxide per hour per sq decimetre under the same conditions as those mentioned above in the case of *Helianthus annuus*. Similar values were found by Willstatter and Stoll for a number of other species.

These values were all obtained under artificial conditions in which a very considerably higher percentage of carbon dioxide was employed than that of the natural atmosphere In experiments under natural conditions it is therefore not surprising that the highest recorded rates of photosynthesis are much lower than those noted above Yet in measurements carried out on rates of photosynthesis in the open air the highest rates have again been recorded for *Helianthus annuus* Sachs, using the half-leaf method, recorded (1884) a gain in weight equivalent to an absorption of carbon dioxide of 16 5 mg per sq decimetre per hour, while Thoday (1910) recorded a still higher rate of photosynthesis for the same species, namely, 19 1 mg per hour, the light being that of bright sunshine in August and the maximum temperature 27 8° C This value is that of the apparent assimilation, if a correction were made for respiration the value of the real assimilation would, of course, be higher. Values obtained for *Catalpa bignonioides* by the same worker were, under the same conditions, only about a third of those obtained with *Helianthus annuus*.

It might be thought that tropical plants would be capable of higher rates of photosynthesis than temperate plants such as *Helianthus annuus*, but this was certainly not the case in experiments performed in the Philippine Islands by F T McLean (1920) The highest rate of photosynthesis recorded by him for sugar-cane leaves on plants in the field is about 5 mg per sq decimetre per hour. This is again a value for the apparent assimilation, so that the actual rate of photosynthesis would be a little higher With leaves of coconut and abaca (*Musa textilis*) the observed rates of photosynthesis rarely reached the neighbourhood of 1 mg per sq decimetre per hour, the rate of apparent assimilation being only about one-sixth of that of sugar-cane leaves under similar conditions

Kostytschew (1922) has concluded that under favourable conditions of carbon dioxide supply Leguminosæ are capable of much higher rates of photosynthesis than are plants belonging to other orders. Thus, using a eudiometric method, he found, for example, that in an atmosphere containing at commencement 8·86 per cent of carbon dioxide exposed at 19 3° C (? to sunlight) in July, *Trifolium repens* assimilated at the rate of about 63 mg per sq. decimetre an hour as compared with about 34 mg per sq decimetre an hour in *Veronica Chamædrys* and *Leucanthemum inodorum* Similar results were obtained with other species But *Alnus glutinosa*, which, like the Leguminosæ, is provided with root nodules, shows an assimilatory activity similar, not to the Leguminosæ, but to plants of other families

CHAPTER VIII

THE PRODUCTS OF PHOTOSYNTHESIS

General Remarks

The substances known without doubt to be produced in assimilating organs as a result of the photosynthetic process are oxygen and carbohydrates The evolution of oxygen from the green parts of plants exposed to sunlight was, as we have seen, one of the first facts in relation to photosynthesis to be recognised That carbohydrates were formed in the assimilating organs at the same time was not realised until very much later when the researches of Sachs (1862*a*, *b*) established this fact Sachs regarded starch as the first visible product of assimilation by showing that it appeared in the chloroplasts after exposure to light and disappeared again in the dark It would appear, however, to be extremely unlikely that so complex and insoluble a substance should be the first actual product of photosynthesis, and direct observation and experiment indicate that there is no doubt that the formation of starch is preceded by the production of simpler carbohydrates Thus, Meyer (1885) found that under the same conditions plants of different species formed different quantities of starch while some formed none at all, as, for example, *Asclepias Cornuti*, and many monocotyledons, especially in the families Liliaceæ, Amaryllidaceæ and Orchidaceæ Again, starch is formed in many plants in the leucoplasts of the root in the dark, a process which cannot be regarded as photosynthetic. Also Kraus (1869) found the first traces of starch appeared in assimilating cells of *Spirogyra* five minutes after the commencement of illumination, whereas it can be shown that evolution of oxygen commences at once

That the products of photosynthesis are either wholly or in great part carbohydrates is indicated by the fact, discussed in the next section of this chapter, that the ratio of oxygen evolved to carbon dioxide absorbed in assimilation very generally approximates to unity (cf the equations on p 44) If other substances, such as fats, organic acids or more complex organic substances, are produced, the ratio would be different Conclusive evidence is derived from chemical analysis of assimilating organs Girard (1884) and Meyer found that in leaves which do not form starch

large quantities of substances capable of reducing cupric solutions are present after exposure to light, while Meyer found there were also present substances which do not reduce such solutions until after hydrolysis. Thus the non-reducing disaccharide sucrose was isolated in crystalline form from vine leaves by Kayser in 1883 [1] Meyer's results were confirmed by Schimper (1885*b*), but the definite establishment of the presence of sugars in the leaf as a result of photosynthesis is due to H Brown and Morris (1893), who pointed out that there was no proof that the reducing substances present in leaves after photosynthesis were actually sugars They therefore tested for various sugars in the leaf of *Tropæolum majus* and found that *d*-glucose, *d*-fructose, maltose and sucrose were present, while pentoses were not found The presence of sucrose and maltose in the leaf extracts of Brown and Morris must be regarded as beyond question, for after treatment of the extracts with invertase the increase in reducing power and change in optical activity is not very different from that which would result if sucrose were hydrolysed to glucose and fructose The presence of maltose was established by obtaining the phenylosazone The presence of glucose and fructose was assumed because glucose phenylosazone was obtained from the leaf extracts, but it is not clear why it was assumed that *d*-mannose, which yields the same osazone as *d*-fructose and *d*-glucose, was absent The analysis of a mixture of sugars is, of course, on account of the close resemblance of so large a number of possible substances, an extremely difficult one Thus, the *l*-forms of glucose, fructose and mannose give a phenylosazone of the same crystalline form and possessing the same melting-point as the *d*-forms of these sugars (see Tollens, 1914) However, no subsequent worker has succeeded in finding any known and recognised hexose sugar present in leaves after exposure to light other than *d*-glucose and *d*-fructose (cf Parkin, 1911 , Davis, Daish and Sawyer, 1916 , Davis and Sawyer, 1916 , Gast, 1917 , Kylin, 1918*b*) Nevertheless, the evidence that no other hexose but these is present is not completely convincing, and there does not appear to be sufficient justification for the statement that "in spite of frequent search it has never been possible to detect *l*-glucose or *l*-fructose in the leaves of plants, and the work of Brown and Morris leaves hardly any doubt that hexoses of the *d*-series and their polysaccharides are the only products of assimilation" On the contrary, Davis and Sawyer (1914) come to the conclusion that pentose sugars are present in leaves, as suggested by the work of de Chalmot (1893*a*, *b*), since in extracts of these there are substances soluble in 80 per cent alcohol which are not precipitated by basic lead acetate, but which reduce cupric solutions and which are not fermented by ordinary yeast There are many

[1] The presence of sucrose in the vine has been confirmed by Davis, Daish and Sawyer (1916), and also by Gast (1917), who drastically criticises the work of Deleano (1912), who thought he had shown the contrary

sugars which possess these properties, the conclusion that pentoses are concerned being deduced from the fact that on distillation with hydrochloric acid according to the Krober-Tollens process (Tollens, 1914) these purified plant extracts give a yield of phloroglucide practically the same as that which would be given by the same weight of pentose calculated as a mixture of *l*-arabinose and *l*-xylose The concordance is, however, not very exact, and Kluyver (1914) has pointed out that the presence of hexoses and disaccharides in such a solution would introduce an error in calculating the quantity of pentose according to the Krober-Tollens method While the production of furfural on distillation with concentrated hydrochloric acid is evidence of the presence of pentose, the presence of other sugars besides those definitely recognised is not ruled out as impossible

Davis, Daish and Sawyer (1916) have thrown doubt on the presence of maltose in leaves They were unable to find this in leaves of mangold, while Davis and Sawyer (1916) could find no evidence of its presence in potato and *Tropæolum* They conclude that the method of preparation of leaf material used by Brown and Morris, consisting of drying the leaves in a steam oven, did not destroy enzymes sufficiently rapidly, and that the maltose found to be present by those investigators arose as a result of enzyme action on starch during the drying of the leaves The same objection is to be raised to the conclusion of Gast (1917) that maltose is present in leaves of *Tropæolum majus, Cucurbita ficifolia, Vitis vinifera, Musa Ensete* and *Canna iridifolia*, for all are starch-forming leaves, and Gast used practically the same method of preparing leaf material as Brown and Morris

Among other carbohydrates and allied substances reported as present in the assimilating organs of various plants are mannitol in Oleaceæ (Meyer, 1886) and in some brown algæ, namely, *Chordaria flagelliformis, Desmarestia aculeata* and *Dictyosiphon hippuroides* (Kylin, 1918*c*), pentosans in mangold (Davis, Daish and Sawyer, 1916), and potato (Davis and Sawyer, 1916) ; dextrin in the potato (Davis and Sawyer, 1916), a dextrin-like soluble carbohydrate in *Laminaria saccharina* and other brown algæ (Kylin, 1915, 1918*c*), and trehalose in the red algæ *Cystoclonium purpurascens* and *Furcellaria fastigiata* (Kylin, 1918*c*) Meyer (1885) reported the presence of sinistrin in leaves of *Yucca filamentosa* but Brown and Morris regarded this as probably inulin Inulin has been found in the leaves of *Cichorium Intybus* by Grafe and Vouk (1912, 1913) and in those of two species of *Marcgravia* by Melchior (1924), and is regarded as an assimulatory product in these plants by these writers According to Kylin (1918*b*), a lævorotatory saccharide accumulates in the leaves of *Gentiana*.

We may conclude, therefore, that in general the commonest carbohydrates to be met with in assimilating organs are starch, sucrose, glucose and fructose. In addition the simpler pentoses may

be present, at least in some cases, while in others various more complex carbohydrates or related substances have been recognised

One of the problems of photosynthesis is the determination of which of these carbohydrates is the one first formed in the leaf which one, that is, that is the actual product of photosynthesis It seems probable that the more complex substances, such as mannitol, trehalose and laminarin, are formed subsequently to the production of some simpler sugar or sugars, while opinion is divided as to whether pentoses are derived from hexoses (De Chalmot 1893*a*, *b*, 1894, Ravenna and Cereser, 1909, Ravenna and Montanari, 1910; Ravenna, 1911, Tollens, 1914), or whether they are formed directly in photosynthesis (Nef, 1910, Löb and Pulvermacher, 1910). In this case they may be formed independently of hexoses as Nef supposed, or they might precede the formation of hexoses as Lob and Pulvermacher thought possible Unless the latter suggestion should be correct, and there is no sound evidence in favour of it, the question of the first carbohydrate to be formed in photosynthesis resolves itself into the problem of determining whether sucrose or hexose sugars are the primary assimilatory products The evidence on this question will be dealt with in a later section of this chapter

There remains the possibility that substances other than carbohydrates are formed in photosynthesis. Briosi (1873) thought that oil, that is, liquid fat, was formed in assimilation by leaves of *Musa* and *Strelitzia*, for he was unable to find starch in these leaves although drops of liquid fat were observed However, this supposition was disproved by Holle (1877) and Godlewski (1877), for the fat droplets do not disappear even during prolonged darkening of the leaves On the other hand, Ewart (1897*b*) found that the drops of liquid fat in the leaves of *Hoya fraterna* only appear after starch has been produced in abundance and disappear before the starch. In the brown algæ vacuoles containing a substance called fucosan (Hansteen, 1892) arise during illumination These vacuoles were examined by Crato (1893) and found to contain phloroglucinol, but a more recent examination of them by Kylin (1918*a*) led this worker to the opinion that the principal content of the vacuoles is a compound closely related to tannin Since the vacuoles are formed under the influence of light Kylin concluded that they must contain products of assimilation, but that the "fucosan" is only to be regarded as a by-product of assimilation Other very problematical supposed assimilatory products, such as the "paramylon" of *Euglena* and other lower plants (Schimper, 1885*a*, Schmitz, 1884, Klebs, 1883) and the "cyanophycin" of the blue green algæ (Palla, 1893) do not call for further mention.

Recently Meyer (1917*a*) has examined the fat-like inclusions occurring in the chloroplasts, especially in those of *Tropæolum*, and has come to the conclusion that the droplets are certainly not composed of a fatty oil The substance composing them increases

in quantity as photosynthesis proceeds and is to be regarded as a product of photosynthesis Subsequently Meyer (1917*c*), from the fact that the aqueous distillate from green leaves contains α β-hexylenealdehyde, claims that this oozes from the assimilatory secretion in the chloroplasts The so-called "oil drops" of *Vaucheria* are similarly supposed by Meyer (1918*b*) not to be true fat, but to be a product of photosynthesis.

The possibility that organic acids may be produced in photosynthesis appears from some fairly recent work of Steinmann (1917). It was found that in rhubarb exposure to light increases the acidity of the expressed sap, while darkening decreases it, and that in general the metabolism of *Rheum* as regards organic acids runs parallel with that of carbohydrates, so that there is a possibility that they are products of photosynthesis and not decomposition products Whether this is so must, however, be regarded as remaining in doubt

The Production of Oxygen

The evolution of oxygen by the green parts of plants exposed to light was established in the latter part of the eighteenth century by Priestley, Senebier and Ingen-Housz, but quantitative data with regard to the amount of oxygen evolved in relation to carbon dioxide absorbed were first obtained by de Saussure in 1804 He recorded his results with plants of five species, namely, *Vinca minor*, *Mentha aquatica*, *Lythrum Salicaria*, *Cactus Opuntia* and *Pinus genevensis* De Saussure found in his experiments that the quantity of oxygen evolved is always less than the carbon dioxide absorbed, and he concluded that "Il résulte de toutes ces expériences, que les plantes, en décomposant le gaz acide carbonique, s'assimilent une partie du gaz oxygène qui y est contenu"

It is a remarkable fact that almost every subsequent writer on this subject has insisted that de Saussure found the volume of oxygen evolved was equal to the volume of carbon dioxide absorbed by the green leaf Thus Sachs (1882, 1887) says, "As an essential point, it is at the same time to be insisted upon here that the volume of oxygen evolved is equal to the volume of the carbon dioxide taken in, as de Saussure and, later and more exactly, Boussingault have already established" Also Palladin (1911, 1923) says, "De Saussure then found that the volume of oxygen given out was equal to that of carbon dioxide taken in." The explanation of de Saussure's result is to be found in the fact that in four out of his five experiments the nitrogen evolved during the experiment was equal to the difference between the carbon dioxide absorbed and oxygen evolved Hence part of the oxygen evolved remains in the intercellular spaces of the tissues, displacing an equal volume of nitrogen which is evolved instead (cf Dehérain and Moissan, 1874)

The ratio of the volume of oxygen evolved to carbon dioxide

absorbed in photosynthesis is called the " assimilatory coefficient," or " assimilatory quotient " It can be denoted by the formula $\frac{O_2}{CO_2}$ (sometimes $\frac{O}{CO_2}$, especially by French writers). A number of writers in German (Kniep, 1912 ; Willstatter and Stoll, 1918) use the term to mean the reciprocal of this value, that is, the ratio of carbon dioxide absorbed to oxygen evolved There appears no reason, either logical or historical, for making this change and introducing this confusion into the nomenclature The term will therefore be used here in its original sense to indicate the relation of oxygen evolved to carbon dioxide absorbed.

If the explanation given above of de Saussure's results is accepted, we may conclude that the assimilatory coefficient in four cases was unity, while in the fifth case it approximated to unity. But in de Saussure's work, as well as in the more exact measurements of Boussingault (1864, 1874), in which also coefficients approximating to unity were found, no account was taken of the contemporaneous process of respiration in the leaf The observed evolution of oxygen is less than that actually formed in assimilation by the amount of this gas used in respiration, while the observed absorption of carbon dioxide is less than that utilised in photosynthesis by the amount of the gas evolved in respiration Only when the respiratory coefficient, the ratio of carbon dioxide evolved to oxygen absorbed in respiration, is unity, will an apparent assimilatory coefficient of unity indicate a real assimilatory coefficient of the same value

An attempt to separate the gaseous exchanges due to respiration and photosynthesis was first made by Bonnier and Mangin (1886*b*), four different methods being employed In the first of these the same green tissue was exposed to darkness and light in a closed vessel and the change in content of carbon dioxide and oxygen in the vessel determined during each of the two periods Then, if in unit time c' and o' are the volumes of carbon dioxide evolved and oxygen absorbed in the dark, and o and c the volumes of oxygen evolved and carbon dioxide absorbed in the light, in unit time, the true assimilatory coefficient is $\frac{o+o'}{c+c'}$

The second method is based on the assumption (see p 119) that by the use of chloroform photosynthesis may be suppressed without affecting respiration By comparison of the gaseous exchange taking place in a quantity of assimilating tissue exposed to light with that occurring in a similar quantity of tissue exposed to the same condition but anæsthetised, the true assimilatory coefficient could be determined

The third method is similar to the second, but assimilation is suppressed, not by an anæsthetic, but by barium hydroxide (cf Garreau, 1851*a*, *b*) This substance is supposed to absorb not only the carbon dioxide present in the atmosphere in the plant chamber, but also the carbon dioxide evolved in respiration,

so that again the difference in the oxygen and carbon dioxide content of the two vessels exposed to the same conditions for the same time gives the true value of the oxygen produced and carbon dioxide utilised in photosynthesis

In the fourth method the gaseous exchanges were measured in green and yellow leaves of the same plant, and it was concluded that the respiration of the non-green leaves so measured is equal to that of the same quantity of the green leaves exposed to the same conditions during the same time.

With the four methods Bonnier and Mangin obtained concordant results They found that the real assimilatory coefficient is always greater than unity, varying between about 1 05 and 1 3. They found the respiratory coefficient always considerably less than unity (0 73 to 0 96), and find in this a reason for Boussingault's determinations always being in the neighbourhood of unity, to which, indeed, their own determinations of the apparent assimilatory coefficient approximate.

There can, however, be little doubt that Bonnier and Mangin's methods are not above criticism. The effect of anæsthetics on respiration is not so simple as assumed (cf. Irving, 1911 , Thoday, 1913*b*), and it would appear extremely doubtful that barium hydroxide would absorb the respiratory carbon dioxide if conditions were such that the leaf could assimilate, while, as Willstatter and Stoll (1918) have shown, yellow leaves may possess considerable photosynthetic activity

Further, Willstatter and Stoll point out that the values of the assimilatory coefficient found by Bonnier and Mangin require a correction on account of the incorrect statement of the experimental data In the cases where this correction has been made the deviation of the coefficient from unity is even greater Values of the coefficient considerably greater than unity were also observed by Schloessing (1892, 1893), Jumelle (1892) for lichens and Jonsson (1894) for mosses Aubert (1892) also obtained similar values for mesophytes, but very much higher values (up to 7 59) for succulents. This is no doubt correlated with the particular metabolism of these plants, the organic acids produced in respiration being further broken down to yield carbon dioxide in the light This carbon dioxide will be utilised in photosynthesis in the green parts, so that the actual quantity of carbon dioxide absorbed from the surrounding medium will be much less than that utilised in photosynthesis and hence much less than the oxygen produced, and so very high O_2/CO_2 ratios are to be expected

A re-investigation of the problem of the assimilatory coefficient by Maquenne and Demoussy (1913) led these workers to dispute the results of Bonnier and Mangin and to conclude that the real assimilatory coefficient approximates to unity Like earlier investigators, they used a closed plant chamber, the change in composition of the gas in which was determined after exposure

to light or darkness Respiratory and apparent assimilatory coefficients were determined from the data so provided, and it was found that the value of the apparent assimilatory coefficient always lay between that of the respiratory coefficient and unity, and as the departure of the respiratory coefficient from unity is never very great in the mesophytic plants examined, they conclude that the real assimilatory coefficient must be still nearer unity than the apparent coefficient From the numbers they obtained experimentally they conclude that the real assimilatory coefficient in the cases they examined never differs from unity by more than $\pm 0{\cdot}01$

In a series of determinations of the assimilatory coefficient, Willstatter and Stoll (1918) used the continuous gas current method, estimating oxygen as well as carbon dioxide They found the value of the real assimilatory coefficient is unity in all the species, exclusive of succulents, they examined Their experiments were made with *Sambucus nigra*, *Pelargonium zonale*, *Cyclamen europæum*, *Æsculus Hippocastanum*, *Ilex aquifolium* (a species which gave a particularly high coefficient in the experiments of Bonnier and Mangin) and the moss *Leucobryum glaucum* In high concentrations of carbon dioxide, in different concentrations of oxygen, in temperatures varying between 10° and 35°, and in high light intensity (about 45,000 lux), and in experiments lasting from 0 5 to 10 hours, the real assimilatory coefficient was always found to be unity. In succulents higher values were found, but with continuous photosynthesis the coefficient progressively approached more nearly 1 Thus, an experiment lasting 0 5 hour gave a value of the assimilatory coefficient of *Opuntia* of 1 5, while over a period of 5 5 hours the value was 1 12. This approximation to unity in long-continued photosynthesis at a high rate suggests that succulent plants exhibit no unusual features in their assimilation, and that the abnormally high assimilatory coefficients observed are completely due to the accumulation of organic acids during respiration in the dark.

More recently Kostytschew (1921*a*) has made estimations of the apparent assimilatory coefficient by a eudiometric method and has found that over very short exposures to light very much less oxygen is evolved than carbon dioxide absorbed, that is, the assimilatory coefficient is decidedly less than unity Over longer periods of illumination the value is unity Thus, in one experiment, leaves of *Betula verrucosa* exposed to sunlight for 3 minutes gave a coefficient of 0 4. Leaves of the same species exposed to direct sunlight for 6 minutes yielded a value of 0 79, while the same leaves exposed for 16 minutes gave an apparent coefficient of unity Similarly, leaves of *Potentilla anserina* exposed to sunlight for 3 minutes gave a value for the apparent assimilatory coefficient of 0 57, but over a period of 43 minutes, a value of 1 00 A mixture of green algæ, *Spirogyra communis* and *Zygnema stellatum*, showed an apparent assimilatory coefficient of 0 21 during exposure to sunlight for 5 minutes, while in another case a value of 0 22 was

found during an exposure of 6 minutes and of 0·79 during an exposure of 21 minutes The low assimilatory coefficients found in experiments of short duration are probably to be explained in relation to the fact that chlorophyll and dead leaf material absorb a certain amount of carbon dioxide, an absorption which in the case of these dead materials does not result in evolution of oxygen The excess absorption of carbon dioxide over evolution of oxygen in the first few minutes of the exposure to the gas in high concentration is probably to be explained as due to a similar absorption by the living leaf, for Willstatter and Stoll (1918) have shown that living leaf material in the dark absorbs considerably more carbon dioxide from an atmosphere rich in that gas than can be accounted for by absorption by the water present in fresh leaves

The First Carbohydrate Product of Photosynthesis

Divergent views are held regarding the first sugar produced in the photosynthetic process. While on general grounds it is to be expected that the simpler hexoses, glucose and fructose, should precede the formation of the more complex disaccharides, several investigators have been led to conclude from their experimental results that sucrose is the first sugar formed in photosynthesis The solution of the problem is difficult for two reasons In the first place, there is the difficulty of chemical analysis already mentioned in the first section of this chapter Secondly, there may clearly be at least four processes in the assimilating organs involving carbohydrate material: the photosynthetic process itself, the transformation of the photosynthetic product into a substance temporarily stored *in situ*, starch in many cases, the transformation of this temporary reserve into a substance capable of diffusion away from the leaf in translocation, and, lastly, the respiratory process involving the breaking down of carbohydrate material Further, carbohydrate may be involved in the formation of more complex organic substances in processes possibly linked in some way with respiration

There are thus quite definitely two series of carbohydrates that may possibly be met with in assimilating organs, those called *upgrade* sugars, formed as intermediate products in the synthesis of, for example, starch, in a leaf that forms that reserve, and *downgrade* sugars resulting from the hydrolysis of the temporary reserve

Evidence on the question of which are the upgrade and which the downgrade sugars has been sought along three lines: (1) by microchemical examination with a view to determining the distribution of different sugars in the leaf; (2) by quantitative determinations of the different sugars present in leaves at different times; and (3) by quantitative determinations of the different sugars in the green and yellow parts of variegated leaves.

Strakosch (1907) investigated the distribution of sugars in the leaf and other parts of the sugar beet by means of microchemical tests depending on the production of osazones The only sugar he was able to find in the mesophyll cells of the leaf was glucose, but in the vascular bundles fructose was found as well, and in the petiole sucrose and maltose Strakosch therefore concluded that the first sugar formed in photosynthesis is glucose, and that sucrose and maltose are formed later This conclusion derived support from analyses of extracts of the leaves and veins, by which it was shown that whereas the leaf extracts contained about six times as much glucose as sucrose, the veins contained nearly five times as much sucrose as hexoses Against Strakosch's results and the similar ones of Van Rytel (1914) is to be cited the opinion of Mangham (1915) that the method used to localise glucose and sucrose, which is due to Senft (1904), is untrustworthy when sucrose is present along with the products of its inversion Also Davis, Daish and Sawyer (1916) state that Grafe's test for fructose (Grafe, 1905) is of doubtful value when glucose is present The same writers also criticise Strakosch's results on the general ground that little reliance can be placed on such microchemical tests for identifying one sugar in presence of another (cf also Ruhland, 1911)

The variations in the carbohydrate content of leaves have been determined in a considerable number of cases, the first systematic research on this subject being the classical investigation of H Brown and Morris on *Tropæolum majus* The carbohydrates in three sets of leaves of this species were determined One set of leaves was picked at 5 a m on August 23 and dried in the steam oven, the second set was picked at the same time and kept in sunshine for twelve hours with the petioles in water and then dried, and the third set was picked at 5 p m after exposure to sunlight for 12 hours The results of the analyses are shown in Table 29.

TABLE 29

CARBOHYDRATE CONTENT OF LEAVES OF *Tropæolum majus* BEFORE AND AFTER INSOLATION

(Data from H Brown and Morris)

Carbohydrate.	Picked and dried 5 a m Percentage of dry weight	Picked 5 a m Kept insolated in water until 5 p m and then dried Percentage of dry weight	Picked and dried 5 p m Percentage of dry weight.
Starch	1 23	3 91	4 59
Sucrose	4 65	8 85	3 86
Glucose	0 97	1 20	0 00
Fructose	2 99	6 44	0 39
Maltose	1 18	0 69	5 33
Total sugars	9 69 [1]	17 18	9 58

Differences in carbohydrate content were also observed between leaves which were picked at once from assimilating plants and

[1] This is the number given by Brown and Morris

from those which had been placed in water in the dark for 24 hours after picking The results of analysis are shown in the following table —

TABLE 30

CARBOHYDRATE CONTENT OF LEAVES OF *Tropæolum majus* BEFORE AND AFTER 24 HOURS IN DARKNESS

(Data from H Brown and Morris)

Carbohydrate	Leaves picked and dried at once Percentage of dry weight	Leaves kept in the dark for 24 hours before drying Percentage of dry weight
Starch	3 693	2 980
Sucrose .	9 98	3 49
Glucose	0 00	0 58
Fructose .	1 41	3 46
Maltose .	2 25	1 86
Total sugars	13 64	9 39

The conclusion drawn by Brown and Morris from these results was that sucrose is the first sugar formed in photosynthesis, and that this also forms a temporary reserve which accumulates as photosynthesis proceeds until, when the concentration has reached a certain value, any further production of sucrose results in the conversion of the excess to starch On translocation from the leaf the sucrose is converted into glucose and fructose, while the starch is hydrolysed to maltose That hexoses are not the first sugars to be formed was thought to be indicated by the fact that after assimilating all day on the plant leaves contained no glucose and very little fructose In cut leaves, where it was supposed that translocation had practically stopped, both sucrose and starch had increased greatly, whereas the amount of glucose had increased only slightly Table 30 shows that in leaves kept with their stalks in water in darkness starch and sucrose decrease considerably, while glucose and fructose both increase considerably. Under these conditions it was supposed by Brown and Morris that loss of carbohydrates occurred chiefly through respiration As presumably the sucrose lost should have given rise to equal quantities of glucose and fructose, and as fructose appears considerably in excess of the glucose, Brown and Morris concluded that the sugar used in respiration is largely glucose

Davis, Daish and Sawyer (1916), however, pointed out several sources of possible error in the methods employed by Brown and Morris That the presence of maltose in the leaf is extremely doubtful has already been pointed out It is also possible that other sugars besides those determined by Brown and Morris are present, and in this case the determination of the quantities of the various sugars, particularly of glucose and fructose, must be regarded as of very doubtful value

Observations on the same lines as those of Brown and Morris have also been made on *Tropæolum majus* and other species by Gast

(1917) Leaves were picked and analysed in the middle of the day and just before sunrise The leaves were dried in an oven at 70° to 80° C., the drying being helped by a strong draught From this material an extract containing the sugars was obtained and the reducing power (and optical rotation) measured (*a*) at once, (*b*) after inversion with invertase from maltase-free yeasts, and (*c*) after fermentation with the maltase-free yeast *Saccharomyces Marxianus* The difference between the first two values gives the value of sucrose, and between the first and third values the hexose, while the third value itself gives the quantity of maltose. From qualitative tests for pentoses it was concluded that these were not present Gast's results for *Tropæolum majus* are shown in Table 31.

TABLE 31

CARBOHYDRATE CONTENT OF LEAVES OF *Tropæolum majus* DURING INSOLATION AND DURING THE NIGHT

(Data from Gast)

Carbohydrate	2 p.m. (summer time), July 24, 1916 Percentage of dry weight		3 a.m. (summer time), July 25, 1916 Percentage of dry weight	
Starch	6 44		5 62	
Sucrose	4 37		2 65	
Maltose	1 07		0 69	
Glucose	0 48	3 76	0 26	2 2
Fructose	3 28		1 95	
Total sugar	9 20		5 55	
Total carbohydrate	15 64		11 17	

From these results, and from rather similar ones obtained with *Cucurbita ficifolia*, *Vitis vinifera*, *Musa Ensete* and *Canna iridiflora*, Gast concluded that there is nearly always more sucrose than other sugars present in leaves, the sucrose content being less in the dark than during assimilation Generally the proportion of sucrose to monosaccharides decreases during the night, although an exception is found in *Canna* Fructose is generally in excess of glucose, which is present in only small amount except in *Musa*. The results thus agree with those of Brown and Morris, except in regard to the quantity of maltose present This was generally very small, *Tropæolum* yielding the highest value of about 1 per cent of the dry weight This result really affords support for the contention of Davis, Daish and Sawyer (1916) that the maltose found by Brown and Morris resulted from hydrolysis of starch during the drying of the leaves, for Gast also used this method of killing his material, but probably carried it out more rapidly The fact that both Brown and Morris and Gast found the quantity of maltose in the leaves varies little during the day and night supports the view of Davis and his collaborators

With regard to the opinion of Brown and Morris that sucrose is the first sugar formed in photosynthesis, Gast prefers to speak

of the "first analytically recognisable sugar" He thinks that both Brown and Morris's experiments and his own point to this sugar being sucrose although they do not prove it And in this connection Dixon and Mason (1916), who regarded hexoses as the primary sugars of photosynthesis, suggest that when a certain concentration of hexoses is reached condensation into sucrose takes place, so that the observed increase in sucrose content as a result of illumination is no reason for regarding this sugar as the first one formed

Parkin (1911) made analyses of the sugars in the leaves of the snowdrop (*Galanthus nivalis*), not only at different times of the day but also at different stages of development of the leaves The analysis is simplified since the snowdrop, like many other monocotyledons, does not form starch in the leaves The leaves were dried at a temperature low enough to prevent discoloration To determine the sugars in the aqueous extract of the dried leaves the reducing power and optical rotation were determined The former is due to hexoses only, the latter to both hexoses and sucrose The reducing power and optical rotation were also determined after inversion with invertase, the increase in reducing power and change in optical activity are due to inversion of the sucrose The quantity of sucrose is thus determined and from the values obtained of reducing power of hexoses and those calculated for the optical activity due to hexoses, the quantities of glucose and fructose were determined Generally no attempt was made to distinguish between the hexoses, and only the amounts of sucrose and total hexose are given

Parkin found that during any particular day in spring the percentage of hexose in the leaf remains fairly constant while the sucrose varies, increasing during the day and decreasing during the night in the way found by Brown and Morris for *Tropæolum* and by Gast for the latter and other species As the season advances the relative proportion of hexose to sucrose in the leaf increases (cf Table 32)

TABLE 32

SEASONAL VARIATION OF CONTENT OF SUGARS IN LEAVES OF *Galanthus nivalis*

(Data from Parkin)

Date of picking	Hour of picking	Maximum shade temperature in Centigrade degrees.	Sucrose in percentage of dry weight.	Hexose in percentage of dry weight	Sucrose / Hexose
Feb 16, 1906	3 p m	9 4	19 8	3 56	1 0 2
,, 26, 1907	4–5 p m	7 2	15 07	2 53	1 0 2
Mar 7, 1906	3 30–4 p m	19 4	14 55	5 69	1 0 2
,, 30, 1905	5–6 p m	9 6	15 5	11 4	1 0 7
Apr 5, 1906 .	4–4 30 p m	15 6	14 64	11 17	1 0 8
,, 5 1907	,,	14 4	14 64	11 61	1 . 0 8
,, 24, 1905	,,	10 6	14 84	17 29	1 1 2
May 4, 1905	3–3 30 p m	11 7	10 3	12 78	1 1 2

Parkin considered his results as affording strong support for Brown and Morris's view that sucrose is the first recognisable sugar to appear in the leaf, and that glucose and fructose are down-grade sugars which arise from the inversion of the sucrose He also found that in 47 cases out of 54 fructose was in excess of glucose, the ratio of fructose to glucose varying from 2 5 to 1 3 In the other seven cases the excess of glucose was only slight, the ratio of fructose to glucose varying from 0 99 to 0 94, and the low value of the ratio is here probably accounted for by experimental error, since in the purification of the extracts there is a greater tendency to destruction of the fructose by careless use of basic lead acetate Parkin thus agrees with Brown and Morris that glucose is used more than fructose in leaf respiration

Work on the sugars of the mangold leaf, which is starch-free except in the earliest stages, was conducted on similar lines by Davis, Daish and Sawyer (1916) Their methods were carefully worked out and tested beforehand (cf Davis and Daish, 1913, 1914; Daish, 1914, Davis and Sawyer, 1914), and on the whole resemble those of Gast, but are more elaborate as they determined pentoses and pentosans as well as sucrose and hexoses Collections of leaves were made from plants growing in the field at 2-hourly intervals over a period of 24 hours, such collections being made on three different dates corresponding to the early, intermediate and final stages of growth Leaves and leaf-stalks were analysed separately, and, in addition, upper and lower parts of the leaf-stalks were analysed separately in the early stage, the midribs of the leaves were analysed separately in the intermediate stage, and in the third stage midribs and leaf-stalks were treated together

The first collections of leaves were made during 24 hours on August 26–27, 1913 Analysis showed that both hexoses and sucrose increase rapidly in amount after daybreak and reach a maximum about mid-day, after which the quantities of each fall regularly and fairly rapidly until the following dawn These changes run closely parallel with temperature changes, and probably also to changes in light intensity. The quantity of sucrose is always greater than that of hexose, while, contrary to the findings of the authors already cited, variations in the quantity of sucrose (1 5 to 3·11 per cent) were found to be relatively less than those in the amount of hexose (0 77 to 2 16 per cent) The quantities of pentose, pentosan and material insoluble in alcohol remain practically constant throughout the day. Slight variations were indeed noted, and Davis, Daish and Sawyer think the variations may have some significance, but it is at present doubtful whether they are due to any other cause than the inevitable differences which occur between different samples of the same material

In the leaves collected at the second stage, during 24 hours on September 10–11, 1912, the hexoses were found to be in excess of the sucrose, and both showed synchronising maxima at 2 p m,

6 p m and 2 a m , but it is possible that these maxima are only the result of sampling differences On the whole both hexose and sucrose increase during the day and decrease during the night. The content of sucrose is about the same as at the earlier stage of growth, but the quantity of hexoses is considerably higher than that found at the earlier date The results obtained for the final stage of growth, during 24 hours on October 11–12, 1912, are on the whole similar to those obtained for the intermediate stage, but the content of hexose is still higher The seasonal variations in carbohydrate content of mangold leaves are summarised in Table 33 The numbers are given as percentages of the dry weight They

TABLE 33

SEASONAL VARIATIONS IN CARBOHYDRATE CONTENT OF MANGOLD LEAVES

(Data from Davis, Daish and Sawyer)

Date	Temperature, in Centigrade degrees	Sucrose	Hexoses.	Pentoses.	Pentosans.
Aug 26–27 .	7 2–23 9	1 50–3 11	0·20–2 16	0·36–0 52	5 19–5 96
Sept 10–11 .	6 1–10 0	4 24–8 27	5 38–8·90	0 34–0 76	4 42–5 90
Oct 11–12 . .	—0 6–16 1	4 98–9 52	9 39–12·41	0 61–0 92	6 21–7 15

show that all the sugars of the mangold leaf increase in quantity as the season advances, and that hexoses form a progressively increasing proportion of the total sugar with advance in the season, as was found to be the case in the snowdrop by Parkin. Also, in the first stage of growth nearly all the hexose and about half the sucrose disappear from the leaf during the night, but as the season proceeds a less proportion of the sugar disappears from the leaf during the night

The midribs and petioles were found always to contain a higher proportion of sugars than the laminæ, and this proportion increases as the season proceeds The hexoses were always found to be much in excess of the sucrose, and the ratio of hexose to sucrose was always found to be much greater in the petioles than in the laminæ Similarly, Parkin found that the sugar content of the snowdrop leaf increases from above downwards and that the hexose : sucrose ratio also increases

From their observations Davis, Daish and Sawyer support the view of Brown and Morris that sucrose is the first sugar formed in photosynthesis, and that this is converted into hexoses for the purpose of translocation The sucrose is supposed to be inverted by the enzyme invertase which is secreted or distributed on the surface of the sieve tubes

A series of observations, similar to those made on the mangold, was made on the potato (*Solanum tuberosum*, var King Edward VII) by Davis and Sawyer (1916) The samples were gathered at 2-hourly intervals on July 16–17, 1914 The potato

leaf contains starch, which was found to reach a maximum at 6 p m , after which it decreased in amount until about 2 a m A quantity of dextrin or soluble starch was present when the starch content reached a maximum value, but it had almost disappeared by about sunset Sucrose was present in considerably higher quantity (about 2 to 3 7 per cent) than hexoses (about 0·5 or less to about 1 3 per cent) Both showed higher values during the day than at night, although the hexose content showed some secondary maxima of doubtful significance

As in the mangold, hexoses were found to be much in excess of sucrose in the leaf-stalks of potato, and, as before, it is reasonable to suppose that sugar is translocated as hexose No maltose was found in spite of the fact that the leaves form starch in abundance. Davis and Sawyer suppose that the enzyme maltase is always present in excess in the leaf, so that the maltose formed in the degradation of starch is always converted at once to glucose Daish (1916) has found maltase present in foliage leaves of a number of different species

Colin (1916-7) also holds that sucrose is the first sugar produced in photosynthesis in the case of the beet, while among earlier workers who held this view are Perrey (1882), Girard (1883, 1884), and Went (1898) Mason (1916) thought, from the results of his work on the carbohydrates of the mosses, that sucrose might be the first sugar formed in photosynthesis in these plants

In the conclusion that sucrose precedes hexose sugars in the assimilating leaf it would appear that two distinct sets of processes have been confused These are the processes of photosynthesis itself leading to the formation of temporary storage products, and the subsequent degradation of these reserves for the purposes of translocation away from the leaf There certainly appears to be evidence pointing to the fact that preparatory to translocation sucrose is converted to hexose sugars Although Peklo (1908), by the employment of microchemical tests, concluded that sucrose predominates in the sieve-tubes of the phloem, the analyses of Davis, Daish and Sawyer appear to bring clear evidence that in the plants they investigated hexoses are in excess of other sugars in the conducting tissues, and the conclusion that sucrose is converted into glucose and fructose by the action of invertase appears reasonable enough, especially as Robertson, Irvine and Dobson (1909) found that enzyme abundant in leaf and stem of the beet, but absent from the root On the ground that the simpler hexoses will diffuse more rapidly than the more complex disaccharide and also because plant cells appear to be in general more permeable to glucose and fructose than to sucrose, we should expect to find sugar translocated as hexoses rather than as sucrose For a diffusion of hexoses to take place from leaf to root we should have a gradient of hexose concentration through the conducting tract. Actually the analyses of Davis, Daish and Sawyer show

generally a higher percentage of hexoses in the petioles than in the leaf veins, which would suggest a flow of hexoses towards the leaf rather than away from it, but there is no definite information with regard to the actual *concentration* of the various sugars in the conducting cells. Indeed, it is doubtful which cells of the vascular tracts constitute the conducting channels of the sugars (cf Dixon, 1923)

While, then, it may be considered likely, although not certain, that sucrose present in the leaf is inverted into glucose and fructose for the purpose of transport, it does not follow, as Davis, Daish and Sawyer appear to suppose, that the sucrose is the first sugar, or even the first analytically recognisable sugar, to be formed as a result of photosynthesis It is quite reasonable to suppose that hexoses are the first formed in *Beta*, and that these are converted to sucrose when a certain concentration is reached, just as in leaves which temporarily store starch this latter product is formed when the concentration of sugar, presumably glucose, reaches a certain concentration

What appears to be more definite evidence with regard to the first sugar produced in photosynthesis is provided by recent work by Weevers (1924), who made determinations of the amounts of sucrose and hexose in the green and yellow parts of variegated leaves of *Acer Negundo, Ilex aquifolium, Hedera Helix, Humulus Lupulus, Euonymus japonica, Æsculus hippocastanum, Cornus sanguinea, Pelargonium zonale, Aspidistra elatior, Chlorophytum Sternbergianum, Ophiopogon jaburan* and *Cyperus alternifolius* In all these both hexoses and sucrose were found to be present in the green parts, whereas in all the species except *Cornus sanguinea* and *Æsculus hippocastanum* sucrose only was found in the yellow parts Moreover, in *Cornus* the amount of hexose in the yellow parts was less than in the green parts of the same leaves, while in *Æsculus* only a small quantity of hexose was found in the yellow parts This limitation of hexoses to the assimilating regions of variegated leaves and the presence of both hexoses and sucrose in the yellow non-assimilating portions afford strong evidence that the hexoses precede the sucrose. In support of this view Weevers records that when leaves of *Pelargonium zonale* are deprived of sugar by a period in the dark and then exposed to light, hexoses are first produced, then sucrose appears, and finally starch

If hexoses are the first formed sugars and sucrose is formed from them, it would appear that not one hexose sugar, but at least two, fructose and glucose, the constituents of sucrose, must be produced in photosynthesis In this connection the work of Nef (1913) on the reciprocal transformations of hexoses in presence of very dilute alkali discovered by Lobry de Bruyn and Van Ekenstein (1895, 1896, 1897), is of particular interest, and the significance of Nef's work from the point of view of carbohydrate synthesis in the plant has been emphasized by Spoehr (1919) Thus an aqueous solution

of *d*-glucose, *d*-mannose or *d*-fructose with 0 05 equivalent of calcium hydroxide at laboratory temperature becomes transformed into an equilibrated mixture containing *d*-glucose, *d*-mannose, *d*-fructose, *d*-pseudofructose and α- and β-*d*-glutose, while hexoses of the *d*-galactose series give a mixture containing *d*-galactose, *d*-talose, *d*-tagatose, *d*-sorbose and α- and β-*d*-galtose No conversion of a sugar from the glucose series to the galactose series takes place

Spoehr points out that although most of the theoretically possible hexoses are now known to the chemist, only a few of these are found in plants By far the most abundant are *d*-glucose and *d*-fructose, the others found at all frequently being *d*-mannose, *d*-galactose and *l*-sorbose Now, in the equilibrium mixtures noted above Nef found aldoses and ketoses present in approximately equal amounts, but in the glucose mixture the only aldoses present were *d*-glucose and *d*-mannose, the former constituting $\frac{5}{6}$ and the latter $\frac{1}{6}$ of the whole aldose, while in the galactose mixture *d*-galactose itself constituted over 90 per cent of the whole of the aldose As Spoehr says, " It is most suggestive that the composition and proportion of the various sugars in equilibrium found in these experiments should so closely approach the conditions existing in nature " While we have no grounds at present for suggesting that such sugar transformations are brought about in the cell by the existence of a definite degree of alkalinity, Nef's work shows that there is no reason to be surprised at the presence of a number of different sugars in the leaf cells, including those that enter into the constitution of the sucrose molecule If one hexose, such as glucose, were the first sugar to be produced in the photosynthetic process, the action of, for example, an enzyme behaving similarly towards the sugars as a weak alkali, would lead to the presence of other hexose or hexoses

Another difficulty presented by the synthesis of sugars in assimilating organs is that the hexoses known to be formed are all optically active and belong to the *d*-series, whereas when an optically active substance is prepared from optically inactive materials in the laboratory an equal quantity of the optical isomeride is produced at the same time. No evidence has yet been produced that *l*-glucose and *l*-fructose occur at all in plants It may be that the *l*-isomerides of the sugars or their precursors are formed and are used in the plant in some unknown way , it is perhaps possible that pentoses present in plants which belong to the *l*-series, and their condensation products the pentosans as well as the pectins into the composition of which pentoses enter, may account, at any rate in part, for the *l*-sugars corresponding to the *d*-glucose, *d*-fructose and sucrose

CHAPTER IX

THE UTILISATION OF ENERGY IN PHOTOSYNTHESIS

General Remarks

It has been pointed out in the introductory chapter that the great significance of the process of photosynthesis for life generally lies in the fact that it involves a transformation of the radiant energy of the sun into the chemical energy stored in the carbohydrate products of photosynthesis Radiant energy is utilised so that compounds of higher energy content are formed from the simpler ones, carbon dioxide and water, present in air and soil

In regard to the utilisation of energy in the photosynthetic process, the first problem to be solved is the determination of the relation between the intensity of the radiation incident on the assimilating organ and the quantities absorbed and utilised by the organ In general, only a portion of the incident energy is absorbed and only a part of the latter is utilised When reliable information with regard to this has been obtained, the next problem will be to discover in what way the absorbed energy is utilised in its transformation into the chemical energy stored in the carbohydrate products of photosynthesis

It should be stated at the outset of a discussion of the energy relations in photosynthesis that our knowledge of the question is very limited Attempts have been made to obtain data with regard to the proportion of the radiant energy incident on the leaf or other assimilating material which is absorbed and utilised in photosynthesis, while various speculations have been made with regard to the way in which the absorbed energy is utilised, but as the experimental data are scanty it is not to be expected that the theoretical speculations rest on any very sure foundation

The problem of the relation between the intensity of the incident radiation and the amount absorbed and utilised is complicated owing to the complex nature of white light In an earlier chapter it has been shown that the rate of photosynthesis is not independent of the wave-length of the incident light, and it cannot be assumed, or even expected, that the utilisation of lights of different wave-lengths will be the same Obviously the relation of the wave-length of the light to its utilisation in photosynthesis must be considered

Determination of the Incident and Absorbed Energy

For the determination of the total radiant energy a number of physical instruments are available, such as the thermopile, bolometer, radiometer and radiomicrometer, for a description of which reference must be made to physical works such as those of Kayser (1900–1912) and Coblenz (1908) The principle involved in all these instruments is the absorption of the radiant energy and its transformation into heat, in which form the total energy can be measured In work on photosynthesis the thermopile and bolometer have been chiefly employed Brown and Escombe (1905*a*) used a pair of differential platinum thermometers, one bright and the other black, as devised by Callendar (1899*a*), the instrument being rendered self-recording by attachment to a recorder devised by the same physicist (Callendar, 1899*b*)

In determining the radiant energy incident on the leaf the measuring instrument must be placed either immediately in front of, or, better, in the exact position occupied by, the assimilating material

The measurement of the quantity of energy absorbed is more difficult Brown and Escombe attempted it in the following manner On a day of bright sunshine the intensity of radiation was measured and the leaf interposed just in front of the measuring instrument The intensity of radiation reaching the instrument was again measured, and the leaf then withdrawn and the value of the total intensity of radiation again determined The mean of the first and third readings was taken as the intensity of total radiation, while the second reading gave the intensity of radiation transmitted by the leaf The difference between the intensity of total radiation and that of the radiation transmitted gave a measure of the absorption of energy by the leaf

In so determining the amount of energy absorbed, the radiation reflected from the surface of the leaf is neglected Brown and Escombe thought this formed only a very small fraction of the total incident energy, but Jorgensen and Stiles (1917) have pointed out that it is extremely unlikely that the reflected radiation constitutes a negligible part of the whole, as a black cloth, for example, may reflect 1 per cent of the incident radiation More recently Warburg and Negelein (1923) have pointed out that when a leaf is interposed between the source of radiation and the measuring instrument, light is scattered in its passage through the leaf, so that a great part of the light issuing from the leaf does not reach the measuring instrument. For this reason they regard the method employed by Brown and Escombe for measuring the energy absorbed as invalid

Warburg and Negelein (1922), who worked with the unicellular alga *Chlorella*, attempted to eliminate the difficulty of measuring the amount of light absorbed by using so thick a suspension of the

alga that all the incident light is absorbed. The suspension of the alga was contained in a trough the sides of which were silvered, so that scattered light should be reflected back into the suspension That absorption of radiation was practically complete in their experiments was shown in two ways Firstly, increase in the thickness of the cell suspension brought about no increase in the amount of photosynthesis in the trough, and secondly, when the pigment from the alga in suspension was extracted with a volume of alcohol equal to that of the suspension and the solution of pigment placed between the source of light and the measuring instrument, the latter gave a reading of zero Warburg and Negelein, however, neglected the light which is reflected from and dispersed through the floor of the trough, as they considered this inappreciable

The energy relations are, of course, more complicated in the assimilating organs of a higher land plant than in an aquatic unicellular alga In the former the total radiant energy falling on the leaf is utilised, as pointed out by Brown and Escombe, in the following ways: (1) in photosynthesis, (2) in transpiration, (3) by transmission through the leaf, and (4) by thermal emission. The last quantity is the energy exchange between the leaf and its surroundings on account of a difference in temperature between the two, so that if the temperature of the leaf is higher than its surroundings, as it usually is, energy will be lost by the leaf to the surroundings, while if the leaf temperature is lower than that of the air surrounding it the leaf absorbs energy from the surroundings It is presupposed that the temperature-difference between the leaf and its surroundings is constant, otherwise energy will be used in raising the temperature of the leaf, and *vice versâ*

In the experiments of Warburg and Negelein the conditions in regard to energy relations are clearly very much simpler. If the assumption that the whole of the incident energy is absorbed by the assimilating cells is correct the energy transmitted is zero, and since no energy is expended in transpiration all the incident energy that is not employed in photosynthesis is transformed into heat This has the effect of raising the temperature above that of the surroundings, with consequent loss of energy from the cells by conduction, a loss of energy which may be included in Brown and Escombe's term "thermal emission" When a condition of equilibrium is reached with regard to temperature so that the cells are maintained at a constant temperature above that of the surroundings, the total incident energy will be equal to the energy used in photosynthesis+the energy lost by thermal emission [1]

[1] There appears to be no foundation for the opinion of Detlefsen (1888) that the energy absorbed by a leaf should be less in air free from carbon dioxide than in air containing this gas

The Determination of the Energy utilised in Photosynthetic Work

The energy utilised in photosynthesis can be estimated from a determination of the amount of carbohydrate product formed However this determination is made the usual correction for respiration must, of course, be applied When a gram of glucose for example, is burnt to carbon dioxide and water, $3\,76 \times 10^3$ gram calories are evolved This quantity is termed the heat of combustion of glucose If the whole of the carbohydrate produced is glucose, the actual transference of radiant energy into chemical energy could be calculated from the quantity of carbon dioxide absorbed or of oxygen evolved, since 44 grams of carbon dioxide give rise to 30 grams of glucose, and consequently 2 56 gram-calories will be expended in the complete assimilation of one gram of carbon dioxide However, as we have seen in the last chapter, glucose is not the only carbohydrate produced in assimilating organs, and the heats of combustion of different substances differ In Table 34 are shown the heats of combustion of a number of organic substances

TABLE 31

Heats of Combustion of a Number of Organic Substances

Substance	Heat of combustion in gram-calories $\times 10^3$
Ethyl alcohol	7 18
Glucose	3 76
Sucrose	3 99
Dextrin	4 1
Starch	4 1
Cellulose	4 2
Leucine	6 5
Vitellin	5 7
Linseed oil	9 47
Olive oil	9 51

In calculating the energy utilised in photosynthesis from the carbon dioxide absorbed, or from the oxygen evolved, we are thus met with the difficulty that we do not know definitely the proportion of the various carbohydrates formed, while, as Table 34 shows, the heats of combustion of the various carbohydrates differ from one another Actual determinations of the heats of combustion of the material produced in photosynthesis have, however, been made by Krasheninnikoff (1901) and Puriewitsch (1914) They measured the increase in dry weight per unit area per hour by the half-leaf method (cf Chapter V), and also the increase in the heat of combustion per unit area per hour The increase in heat of combustion per unit increase in dry weight gives the heat of combustion of the products of assimilation Krasheninnikoff is reported to have obtained a mean value for this of $4\,4 \times 10^3$ gram-calories, while Puriewitsch obtained a value of $4\,4 \times 10^3$ gram-calories for *Acer platanoides*, and values of $5\,2 \times 10^3$ and $4\,5 \times 10^3$ gram-calories for *Polygonum sacchalinense* These values are

considerably higher than the value for glucose, and suggest that other substances besides that particular sugar are formed in the leaf, the values are indeed higher than those for sucrose, starch and cellulose, and if they are of any but a low order of accuracy they point to the production of protein or fat in the leaf during an assimilating period But possibly the divergence between these results and the values for carbohydrates is explicable on account of experimental error

In calculating the energy utilised in photosynthesis a further difficulty arises in any case, because we do not know whether we should regard the photosynthetic process as stopping at the hexose, sucrose or starch stage If either hexose sugar or sucrose were the first product of photosynthesis the production of that particular sugar might be regarded as the end of the photosynthetic process, and the subsequent formation of other carbohydrates would then be regarded as a further reaction not belonging to the photosynthetic process proper It is obvious that in the present state of our knowledge of the course of the photosynthetic action we can only obtain a value of the energy utilisation in the process which must be regarded as an approximate one Brown and Escombe determined the weight of carbon dioxide absorbed by the leaf, and multiplied the value they obtained for this by a carbohydrate factor 0 640 to give the weight of carbohydrate produced This factor they calculated from the analyses of leaves of *Tropæolum majus* made by Brown and Morris, to which reference has been made in the last chapter They then assumed that in the production of each gram of carbohydrate $3\ 76 \times 10^3$ gram-calories are utilised Probably little error is introduced on account of the carbohydrate factor, as variations in the ratio of the carbohydrates present in the leaf and errors in the analysis of Brown and Morris will make little difference to this With regard to the assumption that the heat of combustion of the products is $3\ 76 \times 10^3$, that of glucose, it must be said that this value is probably too low when all the substances formed in the leaf are considered as resulting in the photosynthesis, as a reference to Table 34 and the values obtained by Krasheninnikoff and Puriewitsch suggest

Warburg and Negelein calculated the energy utilised from determination of the oxygen evolved If v c c of oxygen are evolved the energy utilised is assumed to be $v\frac{112300}{22400}$ calories, a value which closely approximates to that used by Brown and Escombe if it is assumed that the whole of the carbon dioxide absorbed is completely transformed with the requisite quantity of water into hexose and oxygen

The Proportion of Incident Energy utilised in Photosynthesis

The Utilisation of Energy in Foliage Leaves—The first and only attempt made to obtain a complete balance-sheet for the leaf in

TABLE 35

ABSORPTION AND UTILISATION OF ENERGY BY LEAVES OF *Polygonum Weyrichii* UNDER VARIOUS CONDITIONS OF INSOLATION

(Data from Brown and Escombe)

Experiment	Date.	Conditions of of experiment	Photosynthesis in c c CO_2 per sq dec per hour	Transpiration in grams per sq. dec per hour	Radiation incident on leaf	Radiation absorbed by leaf	Energy used in photosynthesis	Energy used in transpiration	Energy lost by re-radiation and convection
					in gram-calories per sq cm per minute.				
1	June 29, 1900	Intermittent sunlight without screen . .	3 20	0 599	0 6120	0 3959	0 0026	0 0592	0 3341
2	June 19, 1900	Full sunshine Leaves under thin canvas screen .	3 758	1 054	0 1942	0 1256	0 0031	0 1041	0 0184
3	June 22, 1900	Intermittent sunlight Leaves under thin canvas screen	3 058	0 868	0 1503	0 0972	0 0025	0 0857	0 0090
4	July 3, 1900	Intermittent sunshine with some showers Leaves under canvas screen	2 271	0 517	0 1431	0 0926	0 0019	0 0510	0 0397
5	July 11, 1900	Hot cloudless day Leaves under thin canvas screen	1 479	1 291	0·2418	0 1565	0·0012	0 1275	0 0278

regard to energy was made by Brown and Escombe It has already been mentioned that they supposed the total radiant energy falling on the leaf is utilised in four ways: by photosynthesis, in transpiration, by transmission through the leaf and by thermal emission Their method of determining the total incident energy, as well as their method of determining the energy utilised in photosynthesis and that transmitted through the leaf, has already been indicated To determine the energy used in transpiration the loss of water from the leaf was determined by weighing, and the energy required to bring about the vaporisation of this weight of water at the temperature of the leaf calculated from the heat of vaporisation of water at that temperature. The energy lost by re-radiation, conduction and convection, that is, by emission, is the difference between the total incident energy and the sum of the quantities of energy transmitted, used in photosynthesis and used in transpiration

In Table 35 are summarised the results obtained by Brown and Escombe for five experiments with *Polygonum Weyrichii*, while in Table 36 these results are summarised so as to show what percentage of the incident energy is utilised in photosynthesis and in other ways

TABLE 36

UTILISATION OF ENERGY BY THE LEAF OF *Polygonum Weyrichii*

(Data from Brown and Escombe)

Experiment	Percentage of incident energy used in photosynthesis	Percentage of incident energy used in transpiration	Percentage of incident energy transmitted	Percentage of incident energy lost by emission.
1	0 42	9 67	35 31	54 60
2	1 59	53 60	35 30	9 51
3	1 66	57 01	35 32	6 01
4	1 32	35 64	35 28	27 76
5	0 49	52 72	35 30	11 49

These results of Brown and Escombe indicate that only a quite small proportion of the total incident energy is used for photosynthesis, while this portion is a very variable quantity, varying, in the experiments cited above, from 0·42 per cent to 1 66 per cent Usually transpiration accounts for the expenditure of more than twenty times as much energy as photosynthesis requires Quite similar results with regard to the small fraction of the total incident energy which is utilised in photosynthesis were obtained by Puriewitsch (1914) The total incident energy was measured by the bolometer, while the energy used in photosynthesis was obtained in three cases by direct determination of the increase in the heat of combustion of unit area of the leaf (cf p 164), and in the other cases by calculation The results are summarised in Table 37.

TABLE 37

UTILISATION OF ENERGY BY FOLIAGE LEAVES

(Data from Puriewitch)

Species	Date.	Duration of experiment in hours	Total incident energy per sq cm in gram-calories	Energy used in photosynthesis per sq cm in gram-calories	Percentage of solar energy used in photosynthesis
Acer platanoides	May 30, 1912	6 0	361 03	2 208	0 6
,, ,,	June 2, ,,	5 0	162 59	1 332	0 81
,, ,,	,, 13, ,,	6 0	240 33	6 508	2 7
,, ,,	,, 19, ,,	5 0	202 20	2 630*	1 3
Helianthus annuus	,, 11, ,,	4 5	132 48	5 977	4 5
Polygonum sachalinense	May 31, ,,	1 33	70 85	5 509	7 7
,, ,,	June 3 ,,	3 0	122 33	5 076	4 1
,, ,,	,, 16, ,,	1 83	97 62	2 585*	2 6
,, ,,	,, 17, ,,	2 33	123 18	4 656	3 7
,, ,,	,, 21, ,,	5 0	136 81	4 540	1 1
,, ,,	,, 23, ,,	5 0	177 00	4 514*	2 5
Saxifraga cordifolia	,, 6, ,,	2 33	68 16	3 450	5 0

* The values marked with an asterisk are those found from determinations of heats of combustion, the remaining values in the fifth column were found by calculation

It has already been pointed out that the determination of the energy utilised in photosynthesis can only be an approximation Nevertheless, the results obtained by Brown and Escombe and by Puriewitsch make it quite evident that with high light intensities only a small part of the incident energy is utilised in photosynthesis In a general way it may be said that in the experiments of Brown and Escombe decreasing the intensity of illumination increased the proportion of the incident energy utilised in photosynthesis In a series of experiments these workers performed in which the intensity of incident radiation was varied by means of rotating sectors placed in front of the leaf, it was found that reducing the intensity of illumination always increased the proportion of the incident energy utilised in photosynthesis [1] This result is what would be expected if light were a factor in excess As long as light is the limiting factor, or factor in relative minimum, the rate of photosynthesis, and consequently the energy utilised in the process, will be proportional, or approximately proportional, to the intensity of illumination, but when the light intensity is increased so that it is no longer a limiting factor, or factor in relative minimum, the rate of assimilation will be chiefly dependent on some other factor, and consequently with increase in the intensity of the light

[1] It is, however, doubtful whether the intensity of illumination can be regulated by the use of rotating sectors (Cf Warburg's experiments on intermittent illumination described on p 98)

no further utilisation of the energy takes place, so that with increasing light intensity the proportion of the energy utilised becomes progressively less

The Efficiency of the Photosynthetic System.—Attempts have been made to determine the ratio of the energy utilised in the photosynthetic process to the energy actually absorbed by the chloroplasts On the assumption that these are the whole of the assimilatory apparatus, or, at any rate, the only part of it which absorbs energy, the ratio obtained has been regarded as giving a value of the efficiency of the system Most of the experimental work on this question is of little value Thus Timiriazeff (1903) assumed the energy absorbed by alcoholic extracts of leaves could be taken as a measure of the absorption of light by chlorophyll, but such extracts contain far less chlorophyll than other substances, while the state of aggregation and distribution of chlorophyll in such extracts are probably quite different from those of chlorophyll in a leaf Also Brown and Escombe (1905*a*) compared the absorption of radiant energy by the white and green portions of a leaf of *Negundo aceroides* and attributed the difference to absorption by the chlorophyll Apart from the errors incidental to their method of measuring the amount of energy absorbed (cf p 162), it cannot be assumed that the only difference between the green and white parts of a variegated leaf is in the presence of pigment, as, indeed, the results of Weevers cited in the last chapter indicate Nevertheless, Weigert (1911) used the numbers obtained by Brown and Escombe with *Negundo aceroides* to determine the efficiency of the photosynthetic system of a different species In one particular experiment Brown and Escombe found that it was possible to reduce an intensity of radiation of 0 5 gram-calorie per sq centimetre per minute to 1/12 of that value, that is, to 0 0417 gram calorie per sq centimetre per minute, without bringing about a decrease in the rate of photosynthesis, but that with further reduction in the light intensity the rate of photosynthesis declined Their estimate of the energy used in photosynthesis was 0 0017 gram-calorie per sq centimetre per minute, or, when light intensity is limiting at 0 042 gram-calorie per sq centimetre per minute, 4 1 per cent of the total incident energy From their experiments with the *Negundo* leaf Brown and Escombe concluded that 4 2 per cent of the incident light is absorbed by the chlorophyll From these values Weigert assumed that the efficiency of the chlorophyll system is 4 1/4 2 or 98 per cent , a value which, as Weigert admitted, is surprisingly high It is, however, evident that a result determined from such extremely doubtful data and by applying data obtained from one species to another, can have no value, and if another of the experiments of Brown and Escombe had been chosen in which they found that 4 48 per cent of the total energy received by the leaf was used in photosynthesis, the absurd value of 107 per cent would have been obtained for the efficiency of the

system, while some of Puriewitsch's results would have given still higher values.

The determinations of the efficiency of the photosynthetic process made by Warburg and Negelein (1922, 1923) with the unicellular green alga *Chlorella* are not vitiated by such errors. Their methods of measuring the quantity of absorbed energy and of energy used in the photosynthetic process have already been indicated In their earlier experiments they used a metal filament lamp, and by means of screens of solutions of ferrous sulphate, copper sulphate and aniline dyes, they removed all but yellow and yellow-red rays with a range of wave-lengths extending from 570–645 $\mu\mu$ They used two intensities of incident illumination and found the percentage of the incident energy utilised in photosynthesis was always less with the more intense incident illumination. Hence, if the quantity of incident energy is E, and the energy utilised is U, the ratio U/E, which can be regarded as the efficiency, is less the greater the intensity of the incident energy To obtain a definite value for the ratio, Warburg and Negelein therefore found the ratio for two values of incident radiation, and then by extrapolation from the two values so found, calculated the value for the limiting case where the intensity of the incident radiation, that is, E, is zero Thus, with two intensities of incident radiation of 0 162 and 0 327 calories $\times$ 10^{-4} per sq centimetre per second, the values of U/E were found to be respectively 0·57 and 0 42, which gave for the limiting value when E=0, a value for the ratio U/E of 0 72 The mean value found in twelve such experiments was 0 71 Clearly values found by extrapolation in this way from two other values only, must be liable to very considerable inexactitude Thus, if extrapolation were carried in the other direction, that of increasing light intensity, a value of zero for the efficiency would be obtained in the neighbourhood of an intensity of incident radiation of 10^{-4} calories per sq centimetre per second, whereas from the experiments of Brown and Escombe and numerous other investigators it is clear that in this rather low light intensity photosynthesis may be considerable in other species The unsatisfactoriness of this method of extrapolation was later realised by Warburg and Negelein

The results of Warburg and Negelein agree with those of Brown and Escombe in that with decreasing intensity of incident radiation they show an increasing percentage of the energy utilised in photosynthesis It has already been pointed out that where the light intensity is high this result is to be expected if light is not a limiting factor In the experiments of Warburg and Negelein, however, the light intensity was very low, but it is impossible to say whether some other factor besides light intensity was present in relative minimum. In any case, the high percentage of energy utilised in photosynthesis is worthy of notice The highest value of U/E actually found was 0 72, this was for an intensity of

incident radiation of 0 202 × 10^{-4} calories per sq centimetre per second Generally, with somewhat lower intensities of incident light, the proportion of the incident energy utilised in photosynthesis was found to be less than this Indeed, Warburg and Negelein point out that considerable variations were found in different experiments, and these they ascribe to the different conditions of the cells in the different experiments They state that cells cultivated in high light intensities (for example, at a distance of 7 cm from a 75-watt lamp) utilise a smaller proportion of the incident energy than cells cultivated in lower light intensities (for example, at a distance of 30 cm. from a 75-watt lamp) This agrees with Harder's conclusion that *Phormidium foveolarum* cultivated in weak light assimilates more rapidly in weak light than plants of the same species cultivated in strong light, while plants cultivated in strong light assimilate more rapidly in strong light than plants cultivated in weak light Earlier experiments by von Richter (1912) on algæ support this conclusion, and so do experiments by Lubimenko (1908), Boysen Jensen (1918), Lundegårdh (1921) and Stålfelt (1920, 1921) on land plants to which reference has been made in an earlier chapter Lundegårdh, it will be recollected, considered the result he obtained could be explained on account of differences in morphological structure in sun and shade leaves (cf Stahl, 1882), namely, differences in the ratio of the mass of the chloroplasts to the area of the surface of the intercellular spaces In the case of *Phormidium*, Harder considers an explanation based on morphological differences inadmissible, and concludes that the different behaviour of "sun" and "shade" plants must be referred to chlorophyll content or to some other internal condition But if this internal condition should be the absorbing surface of the chlorophyll-containing bodies, the explanation is essentially the same as that offered by Lundegårdh

The Utilisation of Radiation of Different Wave-lengths in Photosynthesis—That the rate of photosynthesis depends not only on the intensity of the incident light but also on the wave-length of the light has already been noticed in an earlier chapter, where it was shown that lights of the same intensity but of different wave-lengths produce different rates of photosynthesis under otherwise similar conditions The question to be considered at this point is not whether different-coloured lights of the same intensity produce different rates of photosynthesis, but what is the relation between wave-length and the proportion of the *absorbed* radiation that is used in photosynthesis

To compare the effects of different wave-lengths in this respect it is necessary, not only that the intensity of the incident radiation should be the same with the light of different wave-lengths, but that the amount of *absorbed* radiation should be the same As pointed out in an earlier chapter, all the earlier work on the influence

of the wave-length of light on the rate of photosynthesis is of doubtful value because no effort was made to use different-coloured lights of the same intensity The experiments of Kniep and Minder (1909), in which this was done, are vitiated by the unsatisfactory method employed for measurement of the rate of photosynthesis, while, since no steps were taken to ensure that equal quantities of the radiation of different wave-lengths were absorbed, their results cannot yield data for calculating the relative proportions of the absorbed radiations which are used for photosynthetic work

The work of Timiriazeff, Reinke and Engelmann on the influence of the wave-length of light on photosynthesis has been touched upon in an earlier chapter, and it was noted there that these workers all found maximum rate of photosynthesis in the red, while Engelmann found a secondary maximum in the blue From a comparison of the curve between the rate of photosynthesis and wave-length and that of absorption of different wave-lengths by chlorophyll or green leaves, Engelmann (1882*a*) concluded that the energy utilised in photosynthesis in light of different wave-lengths is proportional to the energy absorbed by the chlorophyll, and his oft-quoted formula $E_{abs}=E_{ass}$ is simply an expression of this, and was apparently meant by its author to indicate no more than proportionality between, and not absolute equality of, these two energy quantities Engelmann's view was held apparently by Timiriazeff and Reinke, and, although owing to their methods of experimentation, the work of all these investigators does not justify further discussion here, it is the correctness or incorrectness of Engelmann's equation that the work we now have to consider aims at determining

An attempt to determine the relation between wave-length of light and the ratio of the rate of photosynthesis to the amount of energy absorbed has been made by Wurmser (1920*b*, 1921), the plant used being the marine alga *Ulva lactuca* By the use of screens three coloured lights were obtained, namely, red (750–560$\mu\mu$), green (560–460$\mu\mu$) and violet (460$\mu\mu$—ultra-violet) The intensity of the incident radiation was measured by the rate of destruction of chlorophyll in acetone solution, a reaction which Wurmser (1920*a*) had previously investigated in relation to the three spectral regions used, so that from the rate of decomposition of the chlorophyll he could calculate the intensity of radiation for each of the three coloured lights used To determine the quantity of energy absorbed, Wurmser measured the absorption spectrum of the thallus of *Ulva* by means of the spectrophotometer and so obtained values of the absorbing power of the thallus for each wave-length From these data, and knowing the intensity of the incident radiation, he calculated the quantity of energy of each kind of light absorbed. The rate of photosynthesis was determined by the colorimetric method of Osterhout and Haas

Wurmser's results are shown in Table 38 It appears that

although only a very small amount of green light was absorbed as compared with the absorption of red light, yet, of the absorbed light, the green is utilised in photosynthetic work to about four times the extent of the red, while the violet, which is absorbed to an intermediate extent, is also utilised to an intermediate extent.

TABLE 38

ABSORPTION OF LIGHT OF DIFFERENT WAVE-LENGTHS AND PHOTOSYNTHESIS
(From Wurmser)

Wave-length in $\mu\mu$.	Assimilation, x	Energy absorbed, E	$\frac{x}{E}$
750–560 (red) .	100	100	1 00
560–460 (green)	24	6	4 00
460 downwards (violet) .	80	34	2 35

Wurmser's results have been criticised both on account of his experimental methods, particularly on account of his method of measuring the rate of photosynthesis (Harder, 1923*b*), and his method of calculating the amount of absorbed energy (Warburg and Negelein) Yet his work must be regarded as a considerable advance on that which preceded it

Wurmser, it will be observed, gives his values of the rate of photosynthesis and of the quantity of absorbed energy in arbitrary units The more recent attempt of Warburg and Negelein (1923) to determine the influence of wave-length of the light on the utilisation of the absorbed radiation in photosynthesis has been made by methods which have already been indicated in this chapter, and which allow the calculation of the actual quantities of absorbed energy and of the rates of photosynthesis, and, consequently, of the energy quantities used in the photosynthetic process As in their earlier experiments, they used the green alga *Chlorella*, and as they had previously found that the utilisation of energy by the alga depends on the conditions under which it has been growing, they cultivated it under uniform conditions The highest percentage utilisation of the absorbed energy is obtained when cells have grown for a time in bright light and are then cultivated for a further period in weaker light Their measurements were always carried out with a very low light intensity

By the use of screens and prisms, light of the following wave-lengths was obtained, but of these, those marked with an asterisk were found unsuitable for experimental work on account of the absence of all photosynthesis in them, or because the rate of photosynthesis under the experimental conditions was too low.

Wave-lengths in $\mu\mu$	
*900–800	infra-red
*780–700	long-wave visible red
690–610	red
578	yellow
546 .	green
436 .	blue
*366 . .	ultra-violet

As in earlier experiments, a layer of a suspension of the alga was used of such a thickness that practically all the light incident on the cells was absorbed Tests showed that with the red and yellow light employed probably more than 97 per cent of the incident radiation was absorbed, and with the blue light more than 99 per cent. The green rays are absorbed to a very much less extent than the others, but here probably more than 90 per cent. of the incident light was absorbed by the cells

As before, the intensity of incident radiation was measured by the bolometer and the rate of photosynthesis by Warburg's method Earlier determinations of the ratio of the energy used in photosynthesis to the energy absorbed (the ratio denoted by U/E) showed that this increased with diminishing light intensity, and Warburg and Negelein calculated the value of this for the limiting case of zero light intensity by extrapolation from two determined values They subsequently realised, however, that this cannot give a satisfactory result in absence of knowledge of the equation connecting U and E Consequently, they measured the ratio in the lowest possible light intensities, and if it showed no important variations in this region, they assumed the value so found to be that in the limiting case when light intensity is zero In this way they obtained the following values (Table 39) for the proportion of the absorbed energy used in photosynthetic work for light of different wave-lengths in the limiting case when E=0 The value of U/E is generally denoted by ϕ and in the limiting case by ϕ_0

TABLE 39

UTILISATION OF ENERGY OF DIFFERENT WAVE-LENGTHS IN PHOTOSYNTHESIS BY *Chlorella*

(Data from Warburg and Negelein)

Wave-length in $\mu\mu$.	Proportion of absorbed energy used in photosynthesis in limiting case when absorbed energy=0 (ϕ_0)
660	0 59
578	0 535
546	0 444
436	0 338

The efficiency, it will be observed, decreases with decrease in the size of the wave-length The value found for green light follows this rule, but, owing to possible incompleteness of absorption of the radiation in experiments with green light the value found for this radiation is somewhat insecure

The values recorded in this table are lower than that recorded earlier by the same workers for yellow light, namely, 0 71 This divergence is due chiefly to the fact that the higher earlier value was found by extrapolation, the mean of the highest values actually determined in the earlier experiments being about 0 61, a value very close to that found for red light in the later experiments

It is a point worth noting that chlorophyll fluoresces, not only

in solution, but also in the cell (Stern, 1920, 1921), when illuminated with yellow, green or blue radiation. The fluorescent light produced in this way is red, which is strongly absorbed by the cell and utilised to a greater extent than the original radiation Hence the effect of fluorescence is to increase the proportion of energy utilised in yellow, green and blue light

It is interesting that a similar decrease in the proportion of the absorbed energy utilised in photochemical work with decreasing wave-length has been found in the photolysis of hydrobromic and hydriodic acids, and, according to Warburg and Negelein, is foreseen by the quantum theory of light They calculate that there are required about 4 light quanta in the red and yellow, and about 5 in the blue, to decompose one molecule of carbon dioxide.

Chromatic Adaptation

Connected with the question of the absorption and utilisation of energy of different wave-lengths is that known as " chromatic adaptation " The theory of chromatic adaptation may be regarded as dating from Engelmann's researches on photosynthesis by marine algæ. He pointed out (1883) that the long red waves are more strongly absorbed by sea-water than light of shorter wave-lengths, consequently green algæ are under unfavourable conditions at a depth below the surface of the sea, as the light which reaches them will be devoid of the red rays which the chlorophyll strongly absorbs The presence in red algæ of the red pigment, which absorbs green and blue-green light, was therefore supposed to be of importance in increasing the quantity of radiation the algæ could absorb The formula $E_{abs}=E_{ass}$ expressed the opinion of Engelmann (1884) that the light that was absorbed was that utilised in assimilation The colour of the chromatophores of the red algæ is thus complementary to the colour of their surroundings The idea has been extended to brown algæ, purple bacteria (Engelmann, 1888*b*, Buder, 1919) and higher land plants (Stahl, 1909) In the case of the latter Stahl concluded that the fact that green and infra-red rays are only weakly absorbed can be correlated with the fact that in diffuse light these rays are present in very small quantity, and so in any case could furnish the plant with little energy, while they form so important a part of direct sunlight that they would injure the plant on account of the rise in temperature resulting According to Stahl the leaf pigment thus presents such an absorption curve in relation to the solar spectrum that weak light is used as much as possible and injury from too strong light is avoided But Iwanowski (1914) comes to the conclusion that the leaf is adapted, not to diffuse, but to strong light, and that those red radiations that are most absorbed by the chlorophyll are those which are least active in bringing about its photolysis, while the violet rays, more active in this respect, are chiefly absorbed by the

yellow pigments Ursprung (1917, 1918*d*) also appears to regard the green colour as particularly suitable for absorption of radiations in weak diffuse light

Particular interest was aroused in the question of complementary chromatic adaptation as a result of the work of Gaidukov (1902, 1903*a*, *b*) who found that filaments of *Oscillaria sancta*, a member of the Cyanophyceæ which is violet in diffuse white light, when grown behind a coloured screen changed its colour in the direction of that complementary to the colour of the light in which it was growing Thus, after a few weeks the filaments were found to be greenish in red light, blue-green in yellow light and yellow-brown in blue light As the cultures grew rapidly the final colour was chiefly due to fresh cells Similar results were obtained with *Oscillaria caldariorum*. For further comments on Gaidukov's results, reference may be made to a paper on the subject by Blackman (1904). On transferring the coloured forms to white light there was no change back to the original colour, and Engelmann (1903) thought it might be a case of an inherited acquired character.

Engelmann's experimental method, the bacteria method, has been called in question by N Pringsheim (1885, 1886*b*, *c*, 1887), Reinke (1884*a*) and Timiriazeff (1885*a*), while von Richter (1912), from experiments made with the use of Winkler's method, concluded that it is simply the intensity of the light that determines the distribution of green and red algæ, and that the red pigment of the red algæ plays as small a part in the life of the red algæ as the anthocyanin pigments in land plants, although Kniep (1913) thought that some of von Richter's results favoured Engelmann's view Because of the divergence of the experimental results of Engelmann and von Richter, Wurmser (1921) carried out experiments with a green and a red alga (*Ulva lactuca* and *Rhodymenia palmata*) and found that in green light the red alga assimilated relatively more strongly than the green, but in violet light very feebly Wurmser therefore concludes that the function of phycoerythrin is that of an optical sensitiser, rendering the chloroplasts capable of absorbing very much more green light He agrees with von Richter that the colour is an adaptation to weak light, not light of the colour of the surroundings, for in this case there should be strong assimilation in blue and violet light

That the change in colour of Cyanophyceæ and other plants cultivated in coloured light is a case of chromatic adaptation has also been called in question Boresch (1913, 1919, 1920, 1921*a*, *b*, *c*, 1922) finds that only a few of the Cyanophyceæ show chromatic adaptation as Gaidukov observed it In some cases the colouring can be brought about by shortage of nitrogen or iron *Phormidium foveolarum*, which is normally of an olive colour, becomes bright green in red light, retains its normal colour in yellow light and becomes violet in blue light. The changes in colour are ascribed to the formation of different modifications of the pigment.

Schindler (1913) also found the colour changes in Cyanophyceæ were principally the result of nutritive conditions. Further, the change in colour of the red alga *Porphyra* to green when cultivated in red light (Gaidukov, 1903 *a*, *b*, *c*) was shown by Kneip (1912, 1914) to be the result of injury, and Wurmser and Duclaux (1920) have shown that the green forms of *Chondrus crispus* and *Rhodymenia palmata* found near the surface of the sea also owe their colour to injury, and contain not only no phycoerythrin, but only 0 25 to 0 5 of the chlorophyll contained in the normal red forms, the strong light bringing about a destruction of the pigment.

Critical experiments with *Phormidium foveolarum* have recently been carried out by Harder (1922, 1923*a*) As Boresch found, the plant develops a green colour in red light and a purple colour in blue light In white light the alga is " dark olive-green brownish." Cultures were grown in both intense and weak light of each colour The rates of photosynthesis of material growing under different conditions of illumination were measured during exposure to light of different colours and of different intensities. Harder then found chromatic adaptation to this extent, that when photosynthesis was measured in red and in blue lights of the same intensity, the material that had been grown in red light assimilated more rapidly in red light, and that which had been cultivated in blue light assimilated more rapidly in blue light than in light of the other colour But also it was found that under strong illumination material that had been growing in strong light assimilated more rapidly than material that had been growing in weak light and *vice versâ*, independently of the colour of the light Harder thus agrees partly with Engelmann that each plant he examined was adapted to assimilate most rapidly in that coloured light in which it had grown, while at the same time he asserts that the plants are also adapted to the light intensity in which they are growing, a conclusion which Richter's experiments supported, and which, as we have seen, is supported by the results of a number of recent investigations on higher land plants.

CHAPTER X

THE MECHANISM OF PHOTOSYNTHESIS

General Remarks

In the previous chapters the subjects of discussion have been the facts of photosynthesis obtained by long and laborious experimental researches in the laboratory and field. The work to be considered in this chapter on what has been called the "mechanism" of photosynthesis is on the whole theoretical although experimental results are adduced in support of the theoretical considerations. Our inquiry is now into the way in which photosynthesis is brought about, an inquiry which has attracted the attention of many eminent chemists as well as plant physiologists, no doubt on account chiefly of the fundamental character of the process for all life, and because through photosynthesis the energy of the sun becomes available for use on the earth, and also, perhaps, because in the plant this synthesis is brought about rapidly and at ordinary temperatures and under ordinary conditions of illumination, whereas without the aid of the living plant the synthesis can only be brought about slowly and under conditions quite different from those of the living cell.

As pointed out in the introductory chapter, the essential characteristic of photosynthesis is the building up from carbon dioxide and water of compounds of higher energy content, carbohydrates, the energy for the process being supplied by the radiant energy of the light from the sun. For the synthesis the green chlorophyll pigments are necessary, and possibly also the yellow pigments which always accompany them. In finding an explanation of the photosynthetic mechanism, clearly one of the problems which has to be solved is the manner in which the energy is absorbed in the course of the process, while no explanation can be regarded as satisfactory which does not explain the part played by the pigments.

It has been realised from the time of Liebig that the photosynthetic process could not be a simple chemical reaction, but must be composed of a number of stages. Many different views have been held, and much has been written, concerning the stages in the photosynthetic process and the intermediate substances formed in the course of it. Much of this writing has been

based on very inadequate experimental material, and has hindered rather than advanced the progress of our knowledge of the subject

The photosynthetic process can be looked at from another point of view In the formation of a hexose sugar from carbon dioxide and water, the carbon dioxide is reduced, one atom of oxygen being replaced by two atoms of hydrogen. Various views of the photosynthetic mechanism have been expressed from this aspect. Thus earlier views all regarded the reduction as being effected by light energy, that is, there was supposed to be a photochemical reduction of carbon dioxide; other views have held the reduction to be brought about by photo-electric or electrochemical means, more recently it has been suggested by Warburg (1921, 1922) that the reduction is a purely chemical process, the action of light being to produce a substance which effects the reduction of the carbon dioxide chemically.

If the first of these views should prove correct, and the action of light is to bring about the reduction of the carbon dioxide into a carbon compound with the same single carbon atom in the molecule, it has been urged (cf Meldola, 1906) that the term "photosynthesis" will have to be abandoned, since the building up of the carbohydrate products, the synthetic part of the process, has nothing to do with light. This objection to the term "photosynthesis" has already been discussed in the introductory chapter, and will therefore not be further considered in this place

The evidence actually available from physiological data with regard to the stages in the photosynthetic process will first be considered, before the more theoretical opinions with regard to the assimilatory mechanism are discussed.

The Stages in Photosynthesis

In considering the stages in the process of photosynthesis, the first question which arises is to determine where the process begins and ends In a wide sense we may regard it as starting with the diffusion of carbon dioxide into the cells of the leaf from the outer atmosphere and finishing, as Sachs would have said, with the production of starch In this case the first stage in the process is a purely physical one, diffusion, which has no direct dependence on the light, nor is in any sense a synthesis Then follow stages in the leaf cells leading to the formation of sugars, and ultimately the concluding stage in which the sugar is transformed into starch The last stage clearly is not to be regarded as an essential stage in photosynthesis, for many plants do not form starch, while we know that sugar transported to non-assimilating organs in starch-forming plants is transformed in those organs into starch The transformation of sugar into starch appears, therefore, to be a process for the production of a non-mobile food reserve, its value in the assimilating cell being that it affords a means of removing from the sphere of action the soluble products of photosynthesis,

and so preventing the accumulation of products which, on the general laws of mass and balanced actions, would lead to a cessation of photosynthetic activity. The action appears to be a reversible one, and an enzyme action is clearly indicated. Lundegårdh (1914) regards the action as a very complicated one, depending certainly not only on the concentration of the sugar in the cytoplasm, but also on the quantity of an enzyme, the concentration of which depends on unknown factors. The formation of starch also depends on the temperature (cf Maige, 1924) and possibly on light (cf Gillis, 1923; Reinhard, 1923), but more evidence is required before we can speak definitely on the influence of these conditions According to Henrici (1921*a*) the minimum temperature and light intensity necessary for photosynthesis are lower than those required for starch formation As is well known, the starch always originates in plastids or chromatophores, which must therefore be the seat of the sugar-starch reaction

The limits which we place on the photosynthetic process are, as Schroeder (1917) points out really a matter of convenience Logically, as Schroeder says, we may, with Reinke (1881*c*), regard the first product of photosynthesis as the first substance to arise in the process with a higher energy content than carbonic acid But as there is no information available with regard to the identity of this substance the photosynthetic process may be regarded as ending with the formation of sugar Since, then, we know the first stage in photosynthesis to be the purely physical process of diffusion, our problem is to determine as far as possible the course of events in the assimilating cells in which the carbon dioxide, now in a dissolved condition, is worked up with water into sugar. In a previous chapter it has been pointed out that there is considerable difference of opinion as to whether hexose (glucose and fructose) or sucrose is the first sugar to be produced in photosynthesis, although, for reasons already indicated, the author inclines strongly to the opinion that hexoses precede sucrose

That the carbon dioxide and water are not converted into sugar in a single step is, of course, suggested by the very great difference in chemical constitution between the raw materials and the products of the process Definite indication that the process consists of a photochemical stage (or stages) and a purely chemical (or enzymic) stage (or stages) was provided by the work of Miss Matthaei on the influence of temperature on the rate of photosynthesis. As light is necessary for the whole process to proceed, it is clear that a photochemical action is involved Photochemical actions are, however, characterised by having low temperature coefficients, the value of Q_{10} varying for different reactions between 1 00 and 1 42 (Plotnikow, 1910, 1911), while Miss Matthaei and a number of subsequent workers (cf Chapter VII) have found temperature coefficients for photosynthesis approximating rather to 2, that characteristic of chemical, including enzyme, reactions While

it is inadvisable to lay too much stress on the value of temperature coefficients in determining the nature of an action, the conclusion appears inevitable that photosynthesis cannot be wholly a photochemical or chain of photochemical reactions; some other action with a higher temperature coefficient must also be involved

That the whole photosynthetic process thus contains a photochemical stage and a purely chemical (or enzymic) stage would explain the fact that some observers have found under certain conditions temperature coefficients less than that characteristic of purely chemical reactions, for if there are these two stages with different coefficients the rate of the whole process will depend on the rate of the process which proceeds relatively more slowly, a rate determined by the reacting masses of the substances taking part in the reaction. Thus, if the photochemical stage is relatively the slower, the temperature coefficient of the whole process will be low, approximating to unity, while if the purely chemical reaction is relatively the slower, the relation to temperature of the whole process will be rather that characteristic of chemical reactions.

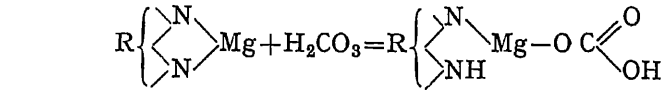
This different behaviour towards temperature was actually observed by Willstatter and Stoll in the case of green and yellow varieties of the same species It was found that the rate of photosynthesis of normal green leaves of *Ulmus*, for example, exposed to strong light (48,000 lux) and in an atmosphere of 4·5 per cent carbon dioxide, was more influenced by temperature than that of leaves of the yellow varieties of the same species Willstatter and Stoll suppose that in the yellow leaves, the chlorophyll being present in comparatively small quantity and being concerned in the photochemical reaction, the photochemical reaction will determine the rate of the whole process, while in green leaves chlorophyll will be in excess and the substances participating in the " dark " chemical reaction will be in relatively smaller quantity than the chlorophyll, and will therefore determine the rate of the whole process, which will therefore show a higher temperature coefficient than in the leaves of chlorophyll-poor varieties. The chemical action Willstatter and Stoll regard as an enzyme action, and, as already noted in an earlier chapter, the enzyme concerned they regard as the protoplasmic factor in photosynthesis

In developing a theory of the photosynthetic mechanism, Willstatter and Stoll (1918, see also Stoll, 1918) suppose that there are actually at least four reactions involved in the formation of sugars from carbon dioxide and water From experiments with living leaves, leaf powder and a colloidal solution of chlorophyll, in which state they believe the chlorophyll to be present in the leaf, they find that chlorophyll is able to absorb a certain quantity of carbon dioxide, with the result that magnesium carbonate and phaeophytin are produced according to the equation (in the case of chlorophyll *a*):

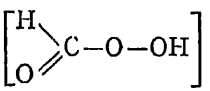
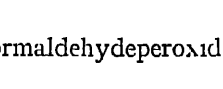
$$C_{55}H_{72}O_5N_4Mg + CO_2 + H_2O = MgCO_3 + C_{55}H_{74}O_5N_4$$

This reaction between carbonic acid and chlorophyll was als observed by Jorgensen and Kidd (1916), and the failure of Kremann and Schniderschitsch (1916) to observe any absorption of carbon dioxide by chlorophyll is attributed by Willstatter and Stoll t their use of impure chlorophyll in unknown quantity and in pre cipitated form

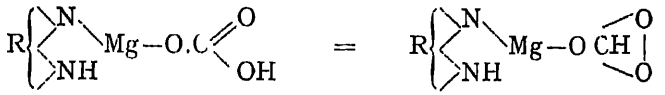
The reaction between chlorophyll and carbonic acid is, however one that proceeds slowly, an intermediate substance being formed which is reversible, so that, under certain conditions, particularly in alcoholic solution, the chlorophyll can be recovered. This intermediate substance is supposed to be an additive compound between chlorophyll and carbonic acid of bicarbonate type, and it is this substance which is supposed to be formed in green cells The reaction may then be represented in the case of chlorophyll a by the equation :



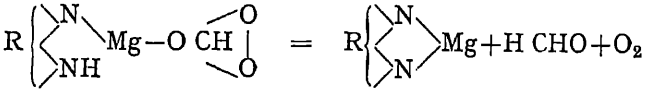
The formation of this additive compound, a purely chemical process is supposed to be followed by the photochemical action which according to Willstatter and Stoll, consists in the molecular re arrangement of the compound into one with higher energy content the energy being absorbed from light The isomer of the chlorophyll-carbonic acid addition compound so formed is regarded as being of peroxide nature, either of the formylhydroperoxide

 or formaldehydeperoxide  type

so that the photochemical stage of the photosynthetic process may be represented by the equation :



This compound is then supposed to undergo decomposition by means of the enzyme regarded by Willstatter and Stoll as the protoplasmic factor of photosynthesis, oxygen being split off with the production of chlorophyll and formaldehyde as a result This stage may be represented by the following equation, although it is possible that the elimination of oxygen may take place in two stages



The reason advanced by Willstatter and Stoll for supposing

that formaldehyde is the intermediate product formed in photosynthesis is that the assimilatory coefficient O_2/CO_2 is unity, whereas, if some other simple compound of carbon, hydrogen and oxygen formed the intermediate product, the coefficient would be smaller. For oxalic acid, for example, it would be 0·25, for formic acid 0 5 and for glycollic acid 0·75 This argument is not convincing, for there appears no adequate reason for supposing that the oxygen must all be given out in one stage If formic acid, for example, were the intermediate substance, half of the oxygen would be evolved in the production of the formic acid, the other half in the formation of sugar The rapidity of the whole process is such that it is impossible to determine in what part of the process the oxygen is evolved, nor has it been found possible to inhibit the latter part of the process so as to obtain an accumulation of the products of the earlier parts of the whole process

The formaldehyde hypothesis of photosynthesis was advanced many years ago, and has been a favourite one. It will be dealt with subsequently in this chapter

The researches of Willstatter and Stoll suggest that there is yet another reaction in the chain of those constituting photosynthesis considered in the wide sense. They found that living leaves in the dark, and leaf powder, absorb many times as much carbon dioxide as can be accounted for by their content of chlorophyll, while the quantities absorbed by green and yellow leaves or leaf material under such conditions are approximately equal. They therefore conclude that there is a mechanism in the leaf for absorbing carbon dioxide, so that the absorption of this substance is accelerated, and its concentration in the assimilating cells raised This absorption can be accounted for by the explanation offered by Siegfried (1905), that carbon dioxide forms definite compounds with amino-acids and proteins which can dissociate again to give the carbon dioxide (see also Spoehr and McGee, 1924).

On the views of Willstatter and Stoll there are thus at least six stages to the whole of the process involved in the formation of sugars in the green leaf These are (1) the diffusion stage ; (2) the absorption of carbon dioxide by plant amino-acids or proteins, presumably a chemical process , (3) the formation of an addition compound between chlorophyll and carbon dioxide , (4) the photochemical isomerisation of this compound , (5) the enzymic splitting off of oxygen and formaldehyde from the isomer with re-formation of the original chlorophyll , and (6) the polymerisation of the formaldehyde into sugar Moreover, it is evident that some of the reactions in the chain may themselves be resolvable into two or more

Much of the theory of Willstatter and Stoll in regard to the details of the stages in photosynthesis rests on a rather slender basis of experimental evidence

A somewhat different view of the matter was propounded by Warburg From his work with *Chlorella* (1919, 1920, 1921, 1922)

Warburg thought that what he called the "photochemical primary action" consisted in the uptake of light energy by the chlorophyll, with the splitting off of oxygen to produce the "photochemical primary product," which then reacts with an "acceptor" which it reduces, the chlorophyll being then re-formed The "acceptor" is not carbon dioxide itself, but a derivative of it produced in the cell by a chain of purely chemical reactions (the so-called "Blackman reaction")

Warburg's view was thus that the actual reduction of the carbon dioxide, or rather its derivative, is purely chemical, the light participating only in producing, by means of the chlorophyll, the reducing substance

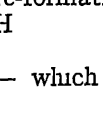
The evidence for Warburg's views was chiefly derived from his experimental results on the influence of carbon dioxide concentration and of light intensity on the rate of photosynthesis It will be recalled that with increasing carbon dioxide concentration above a certain amount Warburg found that the increase in the rate of photosynthesis rapidly falls off until at a certain concentration of carbon dioxide further increase in the latter produces no effect on the photosynthetic velocity Warburg explained this result on the ground that the rate of photosynthesis depends not only on the concentration of carbon dioxide, but on that of some other substance as well with which the carbon dioxide reacts Since the relation of light intensity to rate of photosynthesis is exactly the same as the relation of the concentration of a substance (carbon dioxide) to photosynthetic velocity, it is concluded that concentration of light energy acts like concentration of carbon dioxide, and this is so because of the production of the photochemical primary product" by the action of light on chlorophyll without the participation of carbon dioxide The temperature coefficient of unity, characteristic of photochemical reactions, when the light intensity is low, and the high temperature coefficient characteristic of chemical reactions, found when light intensity is high, can be adduced as an argument in favour of the existence of these two reactions, as in the former case the light action would limit the whole process, and in the latter case the "dark" reaction

Further arguments for this theory were obtained from the action of hydrogen cyanide on photosynthesis of *Chlorella* (cf p 124) Since in light intensities so low that respiration exceeds photosynthesis the rate of the latter is not depressed by concentrations of hydrocyanic acid of M/200 and lower, it is concluded that the photochemical action is not affected by low concentrations of the acid. That a depression of the rate of photosynthesis should occur in high light intensities when very much lower concentrations of hydrocyanic acid are present was explained by Warburg on the ground that the acid inhibits the production of the "acceptor" from the carbon dioxide When the rate of respiration exceeds the rate of photosynthesis respiratory carbon dioxide is not used for photo-

synthesis, but an intermediate product of respiration. That photosynthesis proceeds in this case shows that the third reaction, that between the " photochemical primary product " and the " acceptor," is not adversely affected by the hydrogen cyanide any more than the photochemical reaction, but only the dark reaction in which an active derivative of carbon dioxide is produced, and which, owing to the utilisation of an intermediate respiration product, is not required when light intensity is so low that respiration exceeds photosynthesis

Recently, however, because they find that temperature, hydrocyanic acid and urethanes act in the same way on the release of oxygen from hydrogen peroxide by *Chlorella* in the dark as on photosynthesis in strong light, Warburg and Uyesugi (1924) come to favour the view of Willstatter and Stoll that the Blackman reaction, that is, the chemical reaction that controls the rate of photosynthesis in strong light, is the removal of oxygen from the photochemically produced peroxide compound of chlorophyll.

A view of the stages in photosynthesis somewhat similar to the earlier theory of Warburg has been put forward by Kurt Noack (1920*a*), who lays stress on the fluorescence of the living leaf, and who therefore regards chlorophyll as a photocatalyst, or photodynamically active substance, which transforms radiant energy into chemical energy Accepting the opinion of Straub (1904) that in the illumination of fluorescent dye solutions a formation of peroxide takes place, Noack thinks that the first part of the photosynthetic process can be resolved into three processes: (1) a change of the fluorescent chlorophyll under the action of light and by absorption of oxygen into a peroxide (the " photochemical primary process "), (2) a conversion of carbon dioxide into a peroxide form without the aid of light energy ("acceptor" formation), and (3) a mutual reduction of the two peroxides with evolution of oxygen, the re-formation of chlorophyll and the production of the grouping



which would easily condense to carbohydrates Noack's theory has much in common with one advanced by Woker (1919), to which Noack refers, but according to which light is also effective in producing the peroxide derivative of carbonic acid

Wurmser's view of the stages in photosynthesis is different from those just considered He supposes that there is a substance in the chloroplast which in presence of light absorbs energy, giving a new substance This change may be represented thus:

$$A = A' - x \text{ calories}$$

The product A′ is then transformed back in the protoplasm to A with release of energy, thus:

$$A' = A + x \text{ calories},$$

and it is by means of the energy so liberated that the protoplasm effects the reduction of the carbon dioxide The substance A is not chlorophyll, because, as Wurmser has shown, the relation of the photo-oxidation of chlorophyll to wave-length, the only known photochemical reaction of chlorophyll, is quite different from the relation of photosynthesis to wave-length. The chlorophyll is therefore supposed to act as an optical sensitiser of the substance A, which may be a colourless substance with its maximum absorption in the ultra-violet

Mention may also be made of the suggestions of Osterhout and Haas (1918*a*) as to the stages in photosynthesis. To explain the gradual rise to a maximum value of the rate of photosynthesis on illumination, they put forward two alternative suggestions According to one suggestion sunlight brings about the decomposition of a substance, and the products of this photochemical reaction then catalyse the main photosynthetic reaction Since, however, the rate of the reaction is not constantly increasing, it follows that the rate of production of the catalyst must fall with time It is therefore supposed that the catalyst is produced from a substance which is not renewed as it is decomposed into the catalyst under the influence of light The catalyst will then be formed in the manner characteristic of a monomolecular reaction It is possible that the catalyst is chlorophyll transformed by light from an inactive to an active form

The alternative suggestion is that the amount of photosynthesis corresponds to the amount of a substance P produced under the influence of light by the reaction S→M→P, in which S represents a substance which does not appreciably diminish during the process. While S might represent inactive chlorophyll, M active chlorophyll, and P a derived substance which then combines with carbon dioxide, the authors of the suggestion do not think it profitable at the present time to attempt a more extended discussion of it

Many other theories have been propounded with regard to the stages of the photosynthetic process, and allusion will be made to some of these in a later section of this chapter, where the question of intermediate products is discussed. The theories mentioned above have been chosen because they take account of the fact to which all the physiological evidence points, and on which all physiologists who have considered this problem are agreed, that at least both a photochemical reaction and a purely chemical (or enzyme) action are involved in the process For this reason the considerable number of theories in which a purely chemical explanation of the photosynthetic process is given must be regarded as inacceptable, and equally inacceptable must be considered the theory of Baly, Heilbron and Barker (1921) as at first propounded, according to which photosynthesis consists of two photochemical stages without involving a purely chemical stage It is true that in a later statement Baly (1923) introduced side reactions into the

theory to account for the re-formation of the photocatalytic chlorophyll *a*. He states that " there is little doubt " that the first part of the photosynthetic process takes place in three stages, namely :

1. Chlorophyll A + H_2CO_3 + light = Chlorophyll B + formaldehyde.
2. Chlorophyll B + Carotin = Chlorophyll A + Xanthophyll
3. Xanthophyll + light = Carotin + Oxygen.

This theory of the interaction of the green and yellow pigments in photosynthesis is that put forward by Willstatter and Stoll in 1913, except that formaldehyde was not mentioned and the reduction of xanthophyll to carotin in equation (3) was supposed to be enzymic and not photochemical Willstatter and Stoll, however, put the theory to the test of physiological experiments (1915*a*, *b*, *c*), and as a result concluded that the theory is untenable They state (1918) that " no indication has been found that in the conditions of life in the plant one of the two pigments is transformed into the other, or that through the photosynthetic process one of the two carotinoids is transformed into the other " The theory indeed runs counter to physiological facts The work of Willstatter and Stoll, Warburg and others has shown that when light intensity, carbon dioxide concentration and chlorophyll content are high, an increase in temperature will bring about an increase in the rate of photosynthesis of the order of magnitude to be expected if the whole process is governed by a " dark " chemical reaction Whether the third reaction in the scheme is photochemical or enzymic, an increase in the rate of photosynthesis with constant light intensity can only be brought about by an acceleration of the equation (1), for this is the only one of the three reactions cited which is part of the direct synthetic process This means that with light intensity and carbon dioxide concentration kept constant, an increase in the rate of photosynthesis must depend on an increase in the active mass of the chlorophyll *a* at the expense of the chlorophyll *b* While the speeding up of reaction (2) by temperature might bring this about, it is clear that temperature has no such influence on the relative quantities of the two chlorophylls present in the leaf Indeed, the constancy of the relations between the two chlorophyll components was emphasised by Willstatter and Stoll, Further, there is no evidence of a reaction between chlorophyll *b* and carotin to produce chlorophyll *a* and xanthophyll As Schroeder (1917) so truly observes, " An hypothesis is to be unconditionally rejected that militates against the laws of chemistry, but just as energetically must one be rejected that is incompatible with the experiences of experimental physiology "

The Function of Chlorophyll

The necessity for chlorophyll for photosynthesis is not in doubt, but considerable differences of opinion exist regarding the part played by the pigment The cautious suggestion of Gerland (1871,

1873) and definitely pronounced view of Sachsse (1877) to the effect that chlorophyll is a product of photosynthesis was even regarded as improbable by the latter three years later (1880) from a consideration of the chemical composition of chlorophyll, and therefore needs no further notice here

Nor need Pringsheim's protective theory detain us long Pringsheim (1881*a*, *b*, *c*) thought that light increased the rate of respiration, and that the function of chlorophyll was to act as a protection against light, with the result that assimilation of carbon dioxide is more prominent in green cells than in colourless ones, which, on the theory, were also capable of assimilation if the radiation which favoured respiration was not present Physiological facts are so much in direct contradiction to this theory that it does not require further discussion (*cf* Pfeffer, 1900).

The chlorophylls, as we have seen, have very characteristic physical and chemical properties, and obviously there is a possibility that either the chemical or physical properties, or both, explain the importance of chlorophyll in the photosynthetic process With regard to physical properties, both the chlorophylls have characteristic absorption spectra, while both are fluorescent. Agreement is now general that the chlorophyll absorbs the light energy necessary for photosynthesis, although opinions differ with regard to the relation of the chlorophyll to the carbon dioxide which is ultimately reduced as the result of the energy absorbed by the chlorophyll Objection has been expressed to the view that chlorophyll acts merely as a sensitiser (Molisch, 1906 , Jost, 1908) On this point Benecke (1924) points out that a sensitiser can only increase the sensitivity of a light-sensitive substance to light of frequencies other than those to which it is usually sensitive , it cannot render a non-sensitive substance sensitive to light Thus silver salts are sensitive to light of certain short wave-lengths, and addition of many dyes that absorb red light will render the silver salts sensitive to red light But since the chloroplasts without chlorophyll have no power to decompose carbon dioxide, the green pigment cannot be regarded as a sensitiser in the ordinary sense. But if by the term " sensitiser " one means a substance which transports the energy of the absorbed light to another substance, then chlorophyll can be regarded as a sensitiser It may, however, be argued that we do not really know whether the chloroplast in absence of chlorophyll may not have the power of decomposing carbon dioxide when subjected to light of certain wavelengths The experimental evidence is, however, all against this possibility

Without going further into the purely photochemical question of the mechanism of optical sensitisation, we may assume that the function, or one of the functions, of chlorophyll is to absorb energy which is finally utilised in the decomposition of carbon dioxide or a derivative of it, such as carbonic acid or a bicarbonate. Wurmser

(1921), as we have seen, supposes that the energy absorbed by the chlorophyll is transferred to a substance A which is not carbon dioxide, but some other substance of the chloroplast which then undergoes decomposition into a substance A′, and that on the re-formation of A from this in the colourless protoplasmic part of the plastid the carbon dioxide is reduced chemically by the energy liberated Other workers have thought of a direct transference of the energy to carbon dioxide or a derivative of it Thus Usher and Priestley (1906*a*, *b*, 1911) thought the absorbed light-energy was transferred to the carbon dioxide, which was thereupon reduced to formaldehyde with production at the same time of hydrogen peroxide, and it was supposed that the reaction was similar to that recorded by Bach (1893), in which uranium salts in presence of carbon dioxide and water were thought to give formaldehyde, and which Usher and Priestley themselves confirmed and which more recently was found to take place when colloidal iron was substituted for uranium salts (Moore and Webster, 1913)

Tswett (1911*a*) also thought that the energy was transferred to the carbon dioxide. His view was that chlorophyll, like all fluorescent substances, under the influence of light undergoes a peculiar reversible change associated with intake of energy. When the original molecular form is regained the energy first taken up is radiated out again as luminescent (that is, phosphorescent) light. Tswett supposed that in the case of chlorophyll the phosphorescent rays are specifically absorbed by the carbonic acid. Although carbon dioxide possesses no power of absorption of light within the range of the visible spectrum, it has to be remembered that in the cell the gas is present in an aqueous medium, and that the ions H^+ and HCO^-_3 are present, and that little is known with regard to the absorption spectrum of carbonic acid in solution. The function of chlorophyll is thus thought to be the changing of polychromatic light-energy into monochromatic red light.

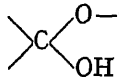
The view of Baly, Heilbron and Barker (1921) is very similar Light is supposed to be absorbed by the chlorophyll (or carotin), and this energy is then radiated out again at infra-red frequencies which are re-absorbed, with the result that carbon dioxide and water are synthesised to formaldehyde and carbohydrates What appears to be a serious objection to this hypothesis is that infra-red light allowed to fall directly on the green cell should bring about photosynthesis if the hypothesis were correct But Warburg and Negelein (1923) record that no photosynthesis was observable in *Chlorella* exposed to infra-red light of wave-length 800 to 900$\mu\mu$, while very little was observed in the longer-waved visible red of wave-length 700 to 780$\mu\mu$ Of course, there is the possibility that infra-red radiation of still higher frequencies might effect the decomposition of carbon dioxide in the chloroplast, although this seems very doubtful Baly, Heilbron and Barker also attach importance to the capacity of the chlorophyll to form a " labile additive

compound" with carbonic acid (Baly, Heilbron and Hudson, 1922) They thus appear to accept the view of Willstatter and Stoll (1918 that chlorophyll plays a chemical part in photosynthesis as well a a physical one But in adopting the earlier view of the Germai workers according to which the light-energy is absorbed only by chlorophyll *a* which is converted into chlorophyll *b* in the photo synthetic process (Baly, 1923), the extreme similarity of the two chlorophylls both physically and chemically appears to be los sight of. Other arguments against this view have been mentioned in the last section of this chapter

As we have already seen, Willstatter and Stoll attribute a very definite chemical rôle to the chlorophyll in addition to its physica function A compound is first formed between the chlorophyl and carbon dioxide, and the action of light is on this compound converting it into a substance of peroxide constitution, which is then acted upon by an enzyme. The light action is, however, not on the chlorophyll part of the molecule, but on the part added to it by the carbonic acid

Other views on the mode of participation of chlorophyll suppose that the action of light is on the chlorophyll itself. Thus, Wager (1914) thought that carbohydrate production in the green cell is "initiated by the photo-oxidation of chlorophyll and subsequen polymerisation of the aldehyde thus formed," the chlorophyl molecule being re-formed by the building up into it of the carbon dioxide. That photo-oxidation of chlorophyll is, however, not the photo-chemical action in photosynthesis is indicated by Wurmser's observations on the relation of photosynthesis and photo-oxidation of chlorophyll to wave-length; moreover, the observations on which Wager's theory were founded are now known to be explic able on other grounds than he assigned (cf Jorgensen and Kidd 1916).

The view of Warburg (1920), in which the chlorophyll is supposed to be acted upon by light to form a "photochemical primary product" that reacts with a derivative of carbon dioxide (the acceptor), has been sufficiently discussed earlier in this chapter Other views, namely, those of Woker (1919) and Kurt Noack (1920*a*, 1921), lay stress on the "photodynamic" action of chloro phyll By a photodynamic action is indicated the action of various substances, including fluorescent dyes and salts of heavy metals which act injuriously on living organisms when illuminated The photodynamic action of chlorophyll was examined by Hausmann (1909), and Woker and Noack, assuming that the photodynami action is due to the production of a peroxide grouping in the fluorescent substance, suppose that the action of light in photo synthesis is to produce a peroxide of chlorophyll Noack, as we have seen, thinks that this peroxide and a peroxide formed from carbonic acid without light action, then mutually reduce with re formation of the chlorophyll, whereas Woker's opinion is that th

chlorophyll first sensitises the bicarbonate in the green cell to form a reactive isomer of peroxide character somewhat of the constitution  . The chlorophyll plays a further part in that under the influence of light the oxygen is withdrawn from the peroxide and produces a chlorophyll peroxide, that is, the isomeric form of the bicarbonate is photochemically reduced.

With regard to the meaning of the presence of two chlorophyll pigments, it has already been noted that Willstätter and Stoll, as a result of their physiological work, rejected their original theory that chlorophyll *a* was transformed in light, after absorption of carbon dioxide and water, to chlorophyll *b* These workers pointed out that the chief absorption bands of chlorophyll *b* lie between those of chlorophyll *a*, so that a mixture of the two pigments will absorb light more completely than one of the components alone, a fact which is of great use to the plant in weak diffuse daylight

A further question which arises, and to which at present no very definite answer can be given, is the function in the assimilating cells of the yellow pigments Willstatter and Stoll (1913) and Ewart (1915) have ascribed a chemical rôle to these pigments, inasmuch as they were supposed to play a part in bringing about the re-formation of the chlorophyll after it had been modified during the photosynthetic process But Willstatter and Stoll, as we have seen, subsequently discarded their original hypothesis on physiological grounds, while the theory of Ewart is clearly untenable on chemical grounds (cf Jorgensen and Kidd, 1916) Willstatter and Stoll (1918) now think that if the carotinoids have any function at all in photosynthesis it must be an indirect one and cannot depend on their absorption of light, as if the leaf is screened from the rays which they absorb, the rate of photosynthesis is not affected appreciably. They think it possible that, having regard to their great power of auto-oxidation, they belong to an arrangement which prevents the photo-oxidation of the chlorophyll.

Warburg and Negelein (1923), however, found that in blue light a considerable fraction of the light absorbed by the pigments of *Chlorella* was taken up by the yellow pigments From their determinations of the ratio of energy used in photosynthesis to the energy absorbed in different regions of the spectrum, they conclude that some of the energy utilised must have been absorbed by the yellow pigments, as if the ratio of energy utilised in chemical work to that absorbed is calculated on the basis of that absorbed by the chlorophyll alone, the ratio is impossibly big Their results point, however, to the fact that a smaller proportion of the energy absorbed by the carotinoids is utilisable for photosynthesis than of that absorbed by the chlorophyll. Historically, it is interesting to note that Engelmann (1887*b*) also thought that the yellow pigments participated in photosynthesis

The Seat of the Photosynthetic Process

It is now generally agreed that photosynthesis takes place in the chloroplasts It is these bodies that contain the chlorophyll, and it is within them that starch is formed in cells that produce this temporary reserve Any attempt to define more accurately the seat of the process or of its constituent reactions must be very much in the nature of a speculation in view of the fact that we have no definite knowledge of the structure of the plastid. It is, however, reasonable to suppose that the chloroplast is a heterogeneous system containing water, proteins and lipoid substances as well as chlorophyll Willstatter and Stoll, as we have already noticed, hold that the chlorophyll is dispersed as a colloidal hydrosol in this system, and Willstatter (1922) speaks of the chlorophyll as being adsorbed in the chloroplast. We might suppose, then, that the photochemical action, at any rate, took place at the surface where the chlorophyll is adsorbed That the chlorophyll is not converted by carbon dioxide into phæophytin is thought by Willstatter and Stoll to be due possibly to the form in which the carbon dioxide is present, and this may be influenced by the presence in the chloroplasts of various substances. Wurmser (1921) holds that the oxygen evolved in photosynthesis cannot be given off from the immediate vicinity of the chlorophyll, as this would be photo-oxidised, and he therefore thinks that a layer of protective colloid surrounds the chlorophyll and prevents oxygen from reaching it Hence he supposes that the photochemical action takes place where the chlorophyll is, and the substance so formed migrates into the stroma away from the chlorophyll, so that the reduction of carbon dioxide and evolution of oxygen takes place away from the chlorophyll Wurmser thus concludes that " La reduction du gaz carbonique a lieu vraisemblablement dans la stroma incoloré des leucites, aux depens de l'energie liberée par une reaction photochimique qui se fait au niveau du pigment."

In relation to Wurmser's views the opinion of Stern (1921) with regard to the condition of the chlorophyll in the chloroplast is of interest (cf p 30). Stern, it will be recalled, thinks the chloroplast consists of an emulsion or emulsion colloid in which a lipoid phase is distributed through an aqueous-protein phase (cf Liebaldt, 1913) The chlorophyll is supposed to be held in true solution in the lipoid particles. Stern thinks that part of the constituent reactions of photosynthesis take part in one phase and part in the other, and in this respect his views accord with those of Wurmser The lipoid substance is supposed to act as a protection against photo-oxidation of the chlorophyll

The importance of the surface between the two phases of the chloroplast is emphasised by Stern. Even if one supposes that the whole chain of reactions takes place in the lipoid phase, the intake of the carbon dioxide or its derivative that is reduced must take

place in the aqueous phase, and the surface separating lipoid and aqueous phases may then be the seat of reactions or merely a zone across which a substance participating in the reactions has to diffuse, and across which an intermediate or final product of the assimilatory process has to pass as well. In any case, only so long as the surface is intact and normal can the photosynthetic process proceed normally A blocking of the surface, as it were, which should result from treatment with surface-active substances which accumulate in the surface and so displace the normal constituents, should have the effect of destroying the normal conditions and so of retarding the rate of photosynthesis.

Such an effect has indeed been observed by Warburg (1919) with surface-active substances, such as phenylurethane, methylurenthane and its homologues, while the retarding effects of other narcotics observed by other workers may also be referred to (cf p 119) Warburg came to the conclusion subsequently proposed by Stern, and supposed that the action of narcotics depends on changes produced in a limiting surface The fact that the relation of rate of photosynthesis to concentration of narcotic gives a curve similar to the adsorption isotherm of Freundlich, supports this view As Stern points out, the fact that Warburg, like previous investigators, found photosynthesis peculiarly sensitive to such substances, much more sensitive, that is, than respiration or irritability, suggests that it is not an ordinary protoplasmic membrane that is involved, but a particular one such as the surface between aqueous protein and lipoid substance might be

In an earlier chapter reference has been made to the work of Briggs (1922*b*) on the influence of a deficiency of nutrient salts on photosynthesis, and it was pointed out that his results led that author to think that the seat of the process is what he calls the " reactive surface of the chloroplast " Although this is not further located, the surface between the aqueous and lipoid phases of the chloroplast postulated by Stern as of such importance in the process of photosynthesis, might correspond to the reactive surface of the chloroplast of Briggs, as Stern's surface would, in fact, be the surface of the phase containing the chlorophyll

Intermediate Products in Photosynthesis

The nature of the intermediate substance or substances formed in photosynthesis is a subject on which much has been written, but on which our real knowledge is practically negligible Many of the theories on this subject have been put forward from the chemical point of view without any consideration whatever of the actual physiological facts The writer can still agree with the comment on this question made by Spoehr in 1916, to the effect that " it can safely be said at the outset that, when critically considered from a physiological view point, none of the existing

theories is even moderately well established by observations (facts "

If this is the case, the very numerous speculations with regar to intermediate products in photosynthesis are not of much intere to the plant physiologist The chief theories that have been pr pounded on this question will therefore be considered only briefl in this place Those who wish for a more detailed account of th theories of photosynthesis should refer to the work of Schroede (1917, 1918) on this subject

The Organic Acid Hypothesis —The view of Liebig (1843*a*) wa that various organic acids were formed as intermediate products i photosynthesis, as, for example, oxalic, tartaric and malic acid Liebig's suggestion was not based on any definite evidence, and i recent years it has found no support Theories approaching i have, however, been suggested by, for example, Ballo (1884), Brunne and Chuard (1886) and Baur (1908), while one particular organi acid, namely, formic, has been more often invoked Reference t these theories will be made later

The Formaldehyde Hypothesis —By far the most favourit hypothesis with regard to the intermediate product in photosynthesi is the formaldehyde hypothesis, which originated from a suggestio thrown out by the chemist Baeyer (1870), who based the idea on a observation previously made by Butlerow (1861) to the effect tha trioxymethylene (a condensation product of formaldehyde) or heating in alkaline medium yields a syrupy substance which ha some of the properties of sugars Baeyer said (translation by Jorgensen and Stiles, 1917): " The general assumption in regard to the formation in the plant of sugars and related bodies is that in the green parts carbon dioxide under the action of light is reduced and by subsequent synthesis transformed to sugar Intermediate steps have been sought in organic acids: formic acid, oxalic acid, tartaric acid, which can be regarded as reduction products of carbon dioxide. According to this opinion, at those times when the green parts of the plant are most strongly subjected to the action of the sun's rays, a strong accumulation of acids should take place, and these should then gradually give place to sugar As far as I know this has never been observed, and when it is remembered that in the plant sugars and their anhydrides are formed under all circumstances, whereas the presence of acids varies according to the kind of plant, the particular part of it and its age, then the opinion already often put forward, that the sugar is formed directly from the carbon dioxide, increases in probability

" The discovery of Butlerow provides the key, and one may indeed wonder that so far it has been so little utilised by plant physiologists. The similarity which exists between the blood pigment and the chlorophyll has often been referred to, it is also probable that chlorophyll as well as hæmoglobin, binds carbon dioxide Now, when sunlight strikes chlorophyll which is surrounded by CO_2,

the carbon dioxide appears to undergo the same dissociation, oxygen escapes, and carbon monoxide remains bound to the chlorophyll The simplest reduction of carbon dioxide is that to the aldehyde of formic acid ; it only requires to take up hydrogen,

$$CO+H_2=COH_2$$

This aldehyde is then transformed, under the influence of the cell contents as well as by alkalies, into sugar As a matter of fact, it would be difficult, according to the other opinion, by a successive synthesis, to reach the goal so easily ! Glycerol could be formed by the condensation of three molecules, and the subsequent reduction of the glyceric aldehyde so formed "

The formaldehyde hypothesis has been put forward in a number of different forms. Reinke (1880, 1881*b*, *c*) thought of the possibility of a direct reduction of carbonic acid, H_2CO_3. Maquenne (1882) suggested methane as an intermediate product between the carbon monoxide and formaldehyde, Bach (1893) thought that hydrogen peroxide was also formed, and Usher and Priestley (1906*a*, *b*) agreed with this view, supposing the hydrogen peroxide is removed by catalase. Pollacci (1902*a*, *b*) and Kimpflin (1908) thought that the reduction of carbon dioxide is brought about by hydrogen, this coming from organic compounds on Pollacci's scheme and from a splitting of water by light action according to Kimpflin (cf also Thunberg, 1923), while Stoklasa and Zdobnický (1911) thought the source of hydrogen was from the enzymic destruction of carbohydrates only. A year later, however, they appear to have abandoned this theory, for in conjunction with Šebor they put forward a scheme in which potassium bicarbonate is acted upon by light to produce potassium carbonate and formic acid which in light is reduced to formaldehyde They thus regard formic acid as an intermediate product between a bicarbonate and formaldehyde

W Lob (1906), from experiments on the effect of the silent electric discharge on carbon dioxide and water, concluded that formaldehyde might be the first product in photosynthesis, or that sugars might be formed without this intermediate substance Baur, to whose theory incident reference has already been made, thought the reduction to formaldehyde might take place in a number of steps through oxalic acid, formic acid, glycollic acid, malic and citric acids He also thought there might possibly be a direct synthesis of sugar from glycollic acid as well as through formaldehyde.

Brunner and Chuard (1886) claimed to have found evidence of the widespread occurrence of glyoxylic acid in the green parts of plants, and they concluded that glyoxylic acid, as well as other acids, glucosides and starch, may all be products of photosynthesis. They thought that from carbonic acid, H_2CO_3, and hydrogen could be obtained —COOH, —CHO and =CO groups which with further hydrogen would give primary and secondary alcohols, formic acid and formaldehyde By interaction of these various groups both

plant acids and hexoses would be produced. The theory has thi points of contact with the organic acid hypothesis of Liebig and th formaldehyde hypothesis of Baeyer Its principal characterist. is, however, that it is a hypothesis of multiple photosynthesis, as involves the production of a number of different photosynthet products

Apart from theories such as that of Willstatter and Stoll (se p 181), where the formation of formaldehyde is rather incidenta than essential to the theory, the experimental evidence that ha been put forward in support of the hypothesis falls under thre heads : (1) The formation of formaldehyde in (*a*) systems containing carbon dioxide and water, (*b*) systems containing carbon dioxide water and chlorophyll, and (*c*) leaves , (2) the formation of suga from formaldehyde ; and (3) feeding experiments with formaldehyde The evidence may therefore be summarised under these heads

(1) *The formation of formaldehyde in various systems* (*a*) There seems no doubt that formaldehyde can be produced from carbon dioxide and water in absence of chlorophyll under certain conditions. Thus, Fenton (1907) found that formaldehyde is produced by the reduction of carbon dioxide by means of metallic magnesium. Lob (1906), as already mentioned, obtained formaldehyde as one of the products when carbon dioxide and water were exposed to the action of the silent electric discharge Several workers have claimed to reduce carbonic acid to formaldehyde by the use of ultra-violet light Berthelot and Gaudechon (1910) claimed that ultra-violet light will effect the splitting of carbon dioxide into carbon monoxide and oxygen and that of water into hydrogen and oxygen In presence of hydrogen they were able to obtain formaldehyde from carbon dioxide by the use of ultra-violet light. Usher and Priestley (1911), by exposing a saturated solution of carbon dioxide in water contained in quartz tubes (which do not absorb the ultra-violet rays) to ultra-violet light, found an easily recognisable quantity of formaldehyde, most of which was polymerised. Stoklasa and Zdobnický (1911) recorded the formation of formaldehyde by the action of ultra-violet light upon carbon dioxide and water vapour in presence of potassium hydroxide and nascent hydrogen More recently Baly, Heilbron and Barker (1921) claim to have reduced carbonic acid to formaldehyde by ultra-violet light This reaction is supposed to be effected by ultra-violet light of very short wave-length (200$\mu\mu$), while ultra-violet light of somewhat longer wave-length (290$\mu\mu$) is supposed to bring about the polymerisation of the formaldehyde to carbohydrate, these being the radiations absorbed by carbon dioxide and formaldehyde respectively The formaldehyde is only produced if the liquid is kept agitated by passing a stream of carbon dioxide through the water, the explanation being that under this condition some of the formaldehyde is carried to the back of the vessel where it escapes the polymerisation brought about by the longer-waved

ultra-violet light. Polymerisation can also be prevented by addition of sodium phenoxide or paraldehyde, which are effective in absorbing the longer waves

Experiments made by Spoehr (1913, 1916) to obtain a reduction of carbonic acid by means of ultra-violet light yielded uniformly negative results, nor could this worker ever obtain any evidence of the formation of formaldehyde when the experiments of Berthelot and Gaudechon, Usher and Priestley, and Stoklasa and Zdobnický were repeated with varying conditions of light intensity, concentration of carbon dioxide and temperature Since the appearance of the paper by Baly, Heilbron and Barker the question was re-examined by Spoehr (1923), but negative results were again obtained However, a repetition of their experiments by Baly and his co-workers (1923) only confirmed their previous findings, and they suggest that the divergence between Spoehr's results and their own might be due to the straight form of quartz mercury vapour lamp used by Spoehr having deteriorated in respect of ultra-violet wave production.

Baly, Heilbron and Barker also report that formaldehyde is produced in their experiments when ultra-violet rays are removed by a plate-glass screen if a basic coloured compound such as malachite green or *p*-nitrosodimethylaniline is added to the water through which the stream of carbon dioxide is bubbled Methyl orange and other substances are reported to bring about the same result.

(*b*) The production of formaldehyde in a system containing water, carbon dioxide and chlorophyll was reported by Pollacci (1902*a*, *b*), Usher and Priestley (1906*a*, *b*), Schryver (1910) and Chodat and Schweizer (1915) On the other hand, Warner (1914) and Ewart (1915) could find no trace of formaldehyde production under these conditions, although in a system containing chlorophyll, water and oxygen, formaldehyde production took place Wager (1914) also found an aldehyde produced under these conditions, but thought it might possibly not be formaldehyde These results suggest that the formation of formaldehyde recorded to take place in systems containing carbon dioxide, water and chlorophyll might be due to the presence of oxygen in the system Moreover, all the experiments were conducted with crude chlorophyll containing much impurity A repetition of the experiments with pure chlorophyll leaves no doubt that the production of formaldehyde was always due to oxidation of the chlorophyll (Jorgensen and Kidd, 1916, see also Willstatter and Stoll, 1918) In systems containing only carbon dioxide, water and chlorophyll no formaldehyde is produced This result militates against the conclusion of Baly, Heilbron and Barker that formaldehyde is produced in such systems exposed to ordinary daylight

(*c*) Many experimenters have recorded the identification of formaldehyde in green leaves after illumination, as, for example, Pollacci (1900–1907), Grafe (1906), Kimpflin (1907), Gibson (1908),

Angelico and Catalano (1913), and Chodat and Schweizer (1913). Formation of aldehyde in illuminated leaves was also observed by Reinke (1881*b*, *c*), Reinke and Kratzschmar (1883) and Curtius and Reinke (1897) Reinke and Braunmuller (1899) and Curtius and Franzen (1912, 1914), held that aldehydes other than formaldehyde are produced, but from the critical researches of Fincke (1913) and Spoehr (1913) there is no doubt that under various conditions aldehydes will be given from a number of substances present in the plant Schroeder (1917), from a review of the experimental evidence, came to the conclusion that there is no indisputable evidence of the presence of formaldehyde in the living green plant (cf also Mazé, 1920, Rouge, 1921, Sabalitschka and Riesenberg, 1924*c*) Experiments of this kind obviously yield no evidence in support of the formaldehyde hypothesis

(2) *The formation of sugars from formaldehyde* —The experiments of Butlerow (1861), Loew (1886, 1887, 1888, 1889*a*), Fischer (1888, 1890*a*, *b*, *c*), Fischer and Passmore (1889), H and A Euler (1906*a*, *b*), Fenton (1907) and particularly Nef (1910, 1913) leave no doubt that various monosaccharides can be synthesised from formaldehyde under certain conditions These conditions are, however, very generally not the same as those existing in the plant, and it is thus impossible to argue from such "in vitro" experiments to the conditions in the living leaf Nor do we know of any enzyme reactions which could bring about this change Baly, Heilbron and Barker have, it is true, reported that the synthesis of formaldehyde to sugar takes place by means of ultra-violet light of wavelength 290$\mu\mu$ (see also Irvine and Francis, 1924), and they state that Benedict's solution will act as a photocatalyst for the synthesis, which, in presence of this solution, will then take place in ordinary visible light They state that chlorophyll is an ideal substance for a photocatalyst for this reaction as well as for the production of formaldehyde from carbon dioxide and water, but they record the results of no experiments in which this supposed function of chlorophyll has been examined.

(3) *Feeding experiments with formaldehyde* —This line of evidence for the formaldehyde hypothesis is based on the assumption that carbon assimilation should proceed in absence of carbon dioxide if an intermediate product be provided as a nutrient Loew (1889*c*) and Bokorny (1888–1911) found that *Spirogyra* in absence of carbon dioxide, but in presence of the sodium bisulphite compound with formaldehyde, forms starch.

Boitreux (1920) found *Trichoderma viride* could utilise formaldehyde, and Moore and Webster (1920) also thought that freshwater algæ can assimilate this substance Grafe and Vieser (1909, 1911), and Miss Baker (1913) held that plants can utilise gaseous formaldehyde in the same way if the concentration of the substance is low enough to prevent its toxic action, and if light is provided In the dark the toxic action alone is observed Spoehr found that

formaldehyde vapour mixed with air is rapidly oxidised to formic acid in sunlight, so that these results could only be used in support of a formic acid theory of photosynthesis

More recently, however, Jacoby (1919, 1922) has asserted that *Tropæolum* leaves can utilise formaldehyde vapour in the dark, and experiments with *Phaseolus multiflorus* and *Pelargonium* made by Sabalitschka and Riesenberg (1924*a*, *b*) favour the same conclusion.

In any case the utilisation of a substance by the leaf is no proof that the substance is an intermediate product in the normal photosynthetic process There are a number of substances which it has been shown can be utilised by green parts of plants in this way (cf Boehm, 1883 , Meyer, 1886 , Acton, 1890), such as glycerol and sugars not normally found in the assimilating organs, but which are not generally supposed to be intermediate products It may also be mentioned that it has not been found possible to utilise carbon monoxide as a nutrient in this way (de Saussure, 1804 , Boussingault, 1868 , Krashéninnikoff, 1909), although according to some forms of the formaldehyde hypothesis this substance is supposed to precede the production of formaldehyde.

From a review of the evidence the writer therefore agrees with Spoehr that the formaldehyde hypothesis, " though alluring on account of its simplicity, is by no means as well established as many writers on the subject would have us believe."

The Formic Acid Hypothesis —Erlenmeyer's view (1877) is a formic acid theory rather than a formaldehyde theory of photosynthesis In comparison with the reduction of α-oxyacids to aldehyde and formic acid, he supposed that carbonic acid is capable of a similar reduction, the products being formic acid and hydrogen peroxide, which would then split into water and oxygen The formic acid might then undergo reduction with formation of formaldehyde and hydrogen peroxide The formic acid theory has since been supported by Phipson (1884*a*, *b*) and others. In the form of the theory as advanced by Wislicenus (1918), after the production of formic acid from carbonic acid by the action of hydrogen peroxide, the formic acid is reduced to formaldehyde in an action which involves light energy and chlorophyll acting as a catalyst This theory is thus both a formic acid and formaldehyde hypothesis The formic acid theory rests on no basis of experimental physiological evidence

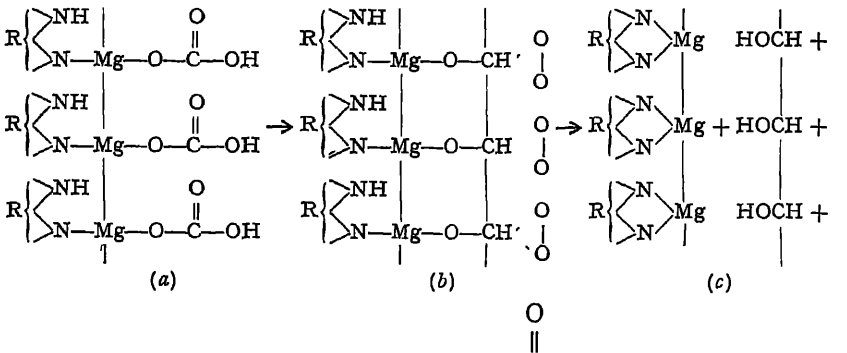
Glycollic Aldehyde Hypothesis —That glycollic aldehyde, $CH_2OH\,CHO$, might be a stage in the formation of sugar from formaldehyde, had been suggested by Reinke (1881*c*), while the view was supported by von Lippmann (1891), but Fincke (1914) thought that glycollic aldehyde might arise directly from carbon dioxide in reduction More recently, Mazé (1920) studied the volatile organic bodies obtained from green leaves that have been actively assimilating, and concluded that formaldehyde never occurs, but that glycollic aldehyde, as well as substances with

the formulæ $CH_3 CHOH CHO$ and $CH_3 CHOH CO CH_3$, was present He concludes (1921) that hydroxylamine plays a fundamental part in bringing about the reduction of carbon dioxide to glycollic aldehyde.

Rouge (1921) has also thought it possible that glycollic aldehyde is the first product in the photosynthetic process He records that tests for the presence of formaldehyde in plant tissue gave negative results, but that, on the contrary, he obtained a yield of 0 012 gram of glycollic aldehyde from 2 kilograms of assimilating potato leaves, while in leaves collected at night only a trace of the aldehyde was found.

Other Hypotheses of the Intermediate Products in Photosynthesis. —This by no means exhausts the theories that have been put forward with regard to the course of the assimilatory process, but the others call for little or no comment As an example may be taken the theory of Pringsheim (1879*b*, 1881*a*), who regarded a substance obtained from green leaves which he called "hypochlorin" as a first product of photosynthesis, but this substance was shown by Meyer (1883*b*) and Tschirch (1884) to be a decomposition product of chlorophyll resulting from the action of acid on an alcoholic extract of chlorophyll and called by Hoppe-Seyler (1879–1881) and Schunck and Marchlewski (1894, 1895) chlorophyllan It is obviously not a product of photosynthesis.

Maquenne (1923) has suggested that there may not be any distinct intermediate product between carbon dioxide and carbohydrate in the assimilatory process He suggests that colloidal chlorophyll, which Willstatter and Stoll suppose to be the pigment in the assimilatory cells, may be produced by the polymerisation of the chlorophyll molecule by the uniting of these molecules through supplementary valences of either nitrogen or magnesium A loose compound between carbonic acid and colloidal chlorophyll may then form, so that the colloidal compound resulting has the following composition (*a*).



The carbon atoms of the attached —O—C—OH groups may then link up with one another, forming a chain parallel with that of the

nitrogen or magnesium atoms of the chlorophyll complex (*b*). By the breaking apart of the two chains and by the loss of two atoms of oxygen from each of the carbonic groupings of the chain of carbon atoms, the characteristic carbohydrate grouping is produced and the chlorophyll complex re-formed (*c*)

The contributions to this section of the subject are thus for the most part highly speculative, and to the writer the trenchant words of Sachs (1882, 1887) written forty-three years ago still seem applicable: "Whether it is right to claim, with Berthelot and Kekulé, formic acid or some other member of the formyl group as the first product of assimilation, on account of its simple constitution, I hold as at least very questionable; and it has hitherto been proved by nothing" The same remarks apply with equal, or even greater force, to other supposed intermediate products in the synthesis of carbohydrates in the leaf.

CHAPTER XI

THE RELATION OF PHOTOSYNTHESIS TO OTHER PLANT ACTIVITIES

General Remarks

In the preceding chapters of this book photosynthesis has been treated very much as if it were an isolated process complete in itself While this mode of treatment is inevitable, it is nevertheless of the greatest importance fully to realise that, just as photosynthesis must be constituted of a chain or complex of reactions, so photosynthesis is only one of a complex of plant activities, all interconnected, which make up the life of the plant Or, put in another way, we can, for the purposes of investigation and description, divide the life activities of the plant into convenient parts which may, to some extent, be arbitrarily chosen, and the limits of which may therefore not always be easily defined Although photosynthesis is one of the most clearly defined of plant activities, we have in preceding chapters met with the difficulty of deciding where the process should be regarded as beginning and ending

That photosynthesis depends on various phenomena which come within the scope of what is called " general physiology," is evident from the facts presented in the preceding pages Thus questions of enzyme action and membrane and cell permeability are clearly involved, as well as phenomena of osmosis and imbibition These questions will not be further discussed here, but in the following sections of this chapter will be presented a very brief review of the relations of photosynthesis to transpiration, translocation and other plant processes

Transpiration

Since water is one of the raw materials used by the plant in photosynthesis, a sufficient supply of water must be maintained in the assimilatory organs It is a possibility that too rapid transpiration might reduce the water content of the assimilating cells to such an extent that water-supply might limit the rate of photosynthesis The question of the relation of the water content of the assimilating cells to photosynthesis has already been dealt with in Chapter VII, and will therefore not be further discussed in this place

The relation between transpiration and photosynthesis is generally thought to be traceable to stomatal action, closure of the stomata beyond a certain degree reduces the rate at which gases will diffuse through them, and consequently both rate of transpiration and rate of photosynthesis will be depressed Consequently, if a plant responds to transference to a dryer atmosphere by closure of stomata, the advantage accruing to it by prevention of excessive evaporation may be accompanied by the disadvantage of a reduced rate of photosynthesis

The problem of the relation between transpiration and photosynthesis has formed the subject of investigation by Iljin (1916) His work had primarily ecological interest, and the results obtained are presented accordingly, while, as he himself realised, his methods are not the most exact It was found that mesophytes in their natural habitats transpire at rates similar to, or slower than, xerophytes in their natural habitats, while the same can be said of the transpiration per unit of carbon dioxide absorbed Transference of mesophytes to a dry xerophytic habitat not only increases the rate of transpiration, but also the rate relative to the rate of photosynthesis Some of the results tabulated by Iljin show that with high rates of transpiration there may be minimal rates of photosynthesis, but it cannot be said that any relation between rate of transpiration and rate of photosynthesis appears from these results

It may be recalled that a deficiency of nutrient salts brings about a reduction in photosynthetic activity (Chapter VII) Transpiration ensures an accumulation of these salts in the cells of the leaf, so it is possible that feeble transpiration long continued might adversely affect photosynthesis on account of the insufficiency of nutrient salts resulting

Translocation and Storage

Little need be added here in regard to the relation of translocation to photosynthesis to what has already been said in Chapter VIII. The products of photosynthesis are, either directly or after temporary storage, translocated away from the assimilatory cells to growing parts or to storage organs Any factor bringing about a cessation or retardation in translocation or storage or cell division, as the case may be, will ultimately lead to cessation or retardation of photosynthesis on account of the accumulation of products in the leaf

Nitrogen Assimilation and Protein Synthesis

The opinion that an intimate relation exists between the production of carbohydrates in green plants and the production of proteins has frequently been expressed, but the evidence on the matter is contradictory The view appeared to be general at one time that in higher plants the synthesis of proteins was confined,

or almost confined, to the leaves (cf. Pfeffer, 1900), and might, indeed, depend on light, while to-day the general opinion appears to be that, although the leaves are the principal centres of protein formation, other organs of the plant possess the power to synthesise this material (cf Czapek, 1920 , Benecke, 1924). There appears no doubt that protein formation is possible in leaves kept in the dark (cf especially Suzuki, 1897*a*, *b*, 1898).

While Meyer (1885) thought the formation of proteins during photosynthesis was not improbable, Saposchnikoff (1890*a*, *b*, 1891, 1893, 1894), because he found the protein content of leaves increased parallel with the carbohydrate content during illumination, thought protein was a product of photosynthesis His results can, however, be interpreted equally well as indicating that protein synthesis depends on a supply of carbohydrate Chibnall's observation (1922) that protein synthesis stops in starved leaves of *Phaseolus vulgaris* var. *multiflorus* containing nitrates, also suggests that protein formation depends on a supply of carbohydrate.

It is outside the scope of this work to enter into a general discussion of the subject of protein synthesis in plants, for an account of which reference may be made to Czapek's summary (1920) of the subject Enough has been said above to make it clear that protein synthesis might be related to photosynthesis of carbohydrates in one of two different ways The nitrogen compounds might be synthesised along with the carbohydrates in reactions coupled in some way with those involved in sugar production, or the proteins and other complex nitrogen compounds might be formed from the carbohydrates themselves which react with nitrates, ammonium salts or some derivatives of these

In favour of the latter view is the undoubted fact that protein synthesis can take place independently of carbohydrate synthesis, since it can occur in the leaves of higher plants in the dark, and in a number of lower plants devoid of chlorophyll On the other hand, experimental evidence suggests that protein synthesis proceeds much more readily in the light than in the dark in leaves of the same species (cf. Godlewski, 1897, 1903 , Wasniewsky, 1914), and it may be that some intermediate product in carbohydrate synthesis, which might also occur as a decomposition product of carbohydrates in other parts of the plant, is concerned in the process Thus, Bach (1896) supposed that nitrate is reduced to nitrite in the plant and ultimately hydroxylamine is formed This reacts with the formaldehyde supposed to be produced in the photosynthetic process and formamide is formed. Various suggestions have been made with regard to the way in which amino-acids might be produced from formamide, but the suggestions are of little interest The view of Baudisch (1911) was rather similar He found that an aqueous solution of potassium nitrite containing methyl alcohol when exposed to ultra-violet radiation gives rise to formhydroxamic acid, and Baudisch supposed that the first steps of protein synthesis

are the reduction of nitrate to nitrite and the reaction of the latter with formaldehyde under the influence of light to produce formhydroxamic acid Baudisch's theory is elaborated in a number of subsequent publications (*e g* Baudisch, 1913, 1916*a*, *b*, 1917, Baudisch and Klinger, 1916) The production of formhydroxamic acid from potassium nitrate or nitrite and formaldehyde in ultra-violet radiation has been confirmed by Baly, Heilbron and Hudson (1922), while none is produced when a solution of potassium nitrite and formaldehyde is allowed to remain in the dark. The reaction in the light, however, takes precedence of the polymerisation of the formaldehyde to sugar, which only takes place when the formaldehyde is produced at a greater rate than that at which it can react with the nitrite present.

Baly and his co-workers lay considerable stress on the readiness with which their formaldehyde produced photochemically (cf Chapter X) reacts with potassium nitrite and polymerises to form reducing sugars, and because of its difference from the " ordinary form " in this respect they speak of it as " activated " formaldehyde They find, further, that activated formaldehyde under the action of ultra-violet radiation can condense with the formhydroxamic acid so formed to give rise to a considerable number of substances present in plants, and they state that " there is no doubt that formhydroxamic acid marks the first step in the photosynthesis of the nitrogen compounds found in the plant " Similar syntheses of nitrogen compounds have been effected by Baly, Heilbron and Stern (1923) with ammonium salts in place of nitrates However interesting and suggestive these observations are, there are various difficulties to be overcome before they can be applied to the synthesis of nitrogen compounds in the plant, chief among which is the difficulty already mentioned, that there seems good evidence that protein synthesis can proceed in the dark, and certainly much more experimental physiological work will have to be performed before we can form any definite opinion with regard to the course of synthesis of complex nitrogen compounds in the plant With this question we are not here concerned, however, so that further discussion of it, and of criticism (Snow and Stone, 1923) of the results of Baly and his co-workers is unnecessary here

Respiration

Since photosynthesis provides the material for respiration, this connection between the two processes is so obvious as to require no emphasis It should, however, be noted that in determinations of carbon assimilation it is usually assumed that respiration proceeds in the assimilating organs at a constant rate It is possible that this assumption is unwarranted If a leaf that has been starved commences to assimilate, the amount of respirable material in it will at first be low, but as photosynthesis proceeds the amount of

this material will be increased Consequently, if the rate of respiration depends on the quantity of respirable material, as is usually assumed (cf. Kidd, West and Briggs, 1921), as photosynthesis proceeds the rate of respiration will also increase The results of Spoehr and MacGee (1923), to which reference has been made in a previous chapter, which indicate that the increase in the rate of respiration during photosynthesis is accompanied by an increase in the rate of photosynthesis, suggest that there is some more subtle connection between respiration and photosynthesis. What this may be can only be discovered by further experimental work.

Vegetative Growth

That vegetative growth depends on photosynthesis is also obvious, since part, at least, of the material used in growth must be provided originally by this process while the energy necessary for the building up of the complex compounds involved must also be secured for the plant in the same process It is interesting to note that realisation of this fact has led a number of workers to inquire during recent years whether the rate of growth of plants can be increased by increasing the carbon dioxide content of the environment of the assimilating organs and so increasing the rate of photosynthesis Although the experiments of Brown and Escombe (1902) on the effect of comparatively high concentrations of carbon dioxide on vegetation indicated that, so far from an increased rate of growth, a stunting of the plants resulted, subsequent experiments of Demoussy (1903) suggested this result might have been caused by impurities in the carbon dioxide employed, for Demoussy obtained a more rapid rate of growth of plants in an atmosphere containing a concentration of carbon dioxide above the normal Subsequently, other workers have recorded an increased rate of growth as a result of increasing the concentration of carbon dioxide in the atmosphere The theory of "carbon dioxide manuring" rests on this basis, that by treatment of the soil with material which will slowly evolve carbon dioxide, or bring about a slow evolution of carbon dioxide from the soil, the concentration of carbon dioxide in the lower levels of the air is maintained above the normal, and increased rate of vegetative growth will result The literature dealing with the influence of high concentrations of carbon dioxide on growth is already a considerable one Reference may be made by those interested to the writings of Demoussy (1904), Fischer (1916, 1920, 1921), Bornemann (1920), Reinau (1920), Lundegårdh (1922*c*, 1924), and further literature cited by Molisch (1918), as a discussion of this work is outside the scope of this book

Reproduction

A connection between photosynthesis and the production of reproductive organs was recognised by Klebs, particularly as a

esult of experimental work on *Sempervivum* He came to the onclusion that the production of reproductive organs in this species s determined by the relation of the rate of production of organic natter in carbon assimilation to the rate of intake of water and lissolved mineral nutrients He summarised his conclusions hus :

" 1. With vigorous carbon assimilation in bright light, strongly ncreased uptake of water and mineral salts results in vegetative growth.

" 2 With vigorous carbon assimilation in bright light, and liminution in the uptake of water and mineral salts, vigorous flower ormation results

" 3 With moderate water and salt uptake, which conditions both ife processes, it depends on the intensity of carbon assimilation which of the two takes place. By reducing the production of organic substance, *e g* in blue light, vegetative growth results, by increasing it, flower formation " (Klebs, 1906)

Again, in a later statement, Klebs (1909) says : " We may call to mind the fact that the formation of inflorescences occurs normally when a vigorous production of organic compounds such as starch, sugar, etc , follows a diminution in the supply of mineral salts On the other hand, the development of inflorescences is entirely suppressed if, at a suitable moment before the actual foundations have been laid, water and mineral salts are supplied to the roots " (see also Klebs, 1918)

While Klebs left it an open question whether all mineral salts or only one in particular may be influential in this way, more recent work suggests that it is particularly the supply of nitrates or other nutrients containing nitrogen that determines, along with the quantity of carbohydrate, whether vegetative or reproductive growth takes place Thus Kraus and Kraybill (1918) came to the conclusion that in the tomato a liberal supply of nitrate, and opportunities for carbon assimilation, lead to vigorous vegetative growth but little fruit, an intermediate supply of nitrates in relation to carbon assimilation leads to intermediate vegetative growth and maximum production of fruit, while a low supply of nitrogen gives little vegetative growth and little fruit , in other words, a low proportion of carbohydrate to nitrogen gives rise to vegetative growth, an intermediate ratio to production of reproductive organs, and a high ratio to poor vegetative growth and poor reproductive growth Confirmatory results were obtained by Gurjar (1920), who found in the same species that the ratio of carbon to nitrogen might vary between 2 and 19, but that fruiting only occurs within the limits of the carbon : nitrogen ratio of 4 and 6 Similar results have been obtained with the apple (Hooker, 1920 , Harvey and Murneek, 1921 , Roberts, 1921) and strawberry (Gardner, 1923), while experiments with other plants, namely, Salvia, buckwheat, radish and Soya bean (Nightingale, 1922), yielded general confirmation

of the theory that formation of reproductive organs is determined by the ratio of carbon to nitrogen in the tissues There thus seems every reason to believe that photosynthesis has a very definite influence, not only on vegetative development, but also in regard to the formation of reproductive organs For further references to the subject of the carbon/nitrogen ratio the writings of Gardner, Bradford and Hooker (1922), Knight (1924) and Summers (1924) may be consulted

CHAPTER XII

CONCLUDING REMARKS

More than forty years ago Reinke (1882) pointed out that the knowledge of carbon assimilation that had been obtained up to that time had been acquired by four distinct methods of research: those of microscopical anatomy, analytical chemistry, experimental physiology and theoretical chemistry. Since then our knowledge of photosynthesis has advanced enormously, and a perusal of these pages will make it clear that many more than four distinct methods have been employed in building up our knowledge of photosynthesis to its present level. Anatomy and histology, supplemented by micro-chemistry, have given us our knowledge of the system involved in the assimilatory organs, while organic chemistry, physical chemistry and colloid chemistry are all involved in the work of determining the composition and state of aggregation of the assimilatory pigments. Special methods of analytical chemistry have had to be devised for the determination of these pigments and of the products of assimilation. Purely physical considerations are involved in determining the mode of entrance of carbon dioxide into the assimilating cells. While methods of physics and chemistry are freely made use of in determining photosynthetic activity, the important work of Blackman and his pupils, of Willstatter and Stoll, as well as of a number of more recent workers, on the influence of internal and external conditions on photosynthesis, and on the utilisation of energy in photosynthesis, has been carried on by the methods of experimental physiology. While the theoretical chemist has not ceased his activities, it is satisfactory to note that the importance of photochemistry has at last been realised, and that suggestive work on photochemical lines has been carried out by more than one worker.

The problems of photosynthesis have thus been attacked by many different methods and from many different points of view, and it is necessary for the advancement of our knowledge of the subject in a balanced way that it should be so. In the past it has clearly often been a weakness of the investigator employing one particular method of attack that he should neglect or minimise the results achieved along other lines of work, as a perusal of the literature dealing with theories of the mechanism of photosynthesis

will make clear This weakness of many an investigator, perhaps a very natural weakness, is still sometimes exhibited, and perhaps will always continue to be, but it is for the plant physiologist to judge the value of the enthusiastic statements of such writers, to take the facts at their true value and to judge the theories in the light of the whole of the available evidence derived from work done, not on one line only, but along all possible lines that have been explored.

While it is not always easy to compare the relative positions of knowledge in different parts of a subject, it may be said, at least, that our knowledge of photosynthesis is as great as that of any other plant process The main facts have been clear for more than half a century, but during the last thirty years many things, once obscure, have been explained, while during the last decade our knowledge has increased materially in many important respects We now understand, thanks to the researches, principally, of Brown and Escombe, how it is that carbon dioxide, present in the air in such low concentration, can enter, through such a restricted path as that presented by the stomata, sufficiently rapidly into the assimilating cells to account for observed rates of photosynthesis Owing to Blackman, Willstatter and others, we now possess a fair knowledge of the relation between the various external and internal conditions and photosynthetic activity Considerable information is now available with regard to the products of assimilation, while a commencement has been made in obtaining data with regard to energy relations in photosynthesis

Much, however, remains to be done. Our knowledge of the protoplasmic factor in photosynthesis is still slight and we are yet without any sufficient evidence of the way in which the assimilatory pigments participate in the processes of carbohydrate production, and particularly the presence of four, and in some cases more, of these pigments calls for explanation Further data are still required with regard to the action and interaction of the various external factors, while an extension of experimental work to species other than those already used is desirable. Our knowledge of the energy relations in photosynthesis is still very slight.

At the present time the chief line of investigation in photosynthesis appears to be the determination of the inter-relations between the internal factors and of the mode of co-operation between internal and external factors This line of work has already led to suggestive results with regard to the course of the assimilatory process. Internal factors are, however, difficult to estimate quantitatively and still more difficult to control. These internal factors operative at any time are a product of hereditary factors and past and present environment factors It seems, therefore, possible that application of the principles of genetics and of developmental physiology may lead to the possibility of controlling the internal factors, in which case the experimenter would be able to

control the rate of photosynthesis of any particular plant species through control of all external and internal conditions.

From the point of view of pure plant physiology a vast field of investigation waits to be explored in regard to the relation of photosynthesis to other plant processes, and so to the life of the plant in general Beginnings have been made in work along this line, but our information at present, although suggestive, is scanty

Finally, it should not be forgotten that the study of photosynthesis has not only a purely scientific interest It is more than likely that the application of scientific results obtained in regard to this subject may be of very considerable importance in agriculture and horticulture, while elucidation of the physiology of the process may provide the key to a means of utilising solar energy for the use of man. As pointed out in the introductory chapter, it is as a result of the storage of the energy of the sun in plants by photosynthesis in past ages that our present industrial development has been able to take place But the supply of energy from coal is not inexhaustible, while other natural sources of energy at present available are probably inadequate to replace coal The utilisation of the radiant energy of the sun is clearly indicated as the means of obtaining a supply of energy available for the maintenance and development of our present civilisation One obvious method is the production of plants in which photosynthesis is rapid, so that material of high calorific value is produced comparatively rapidly Whether this means, or the direct storage of the energy of sunlight by some photochemical reaction, will be the method ultimately adopted, is a question which at present is outside the limits of profitable speculation

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